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Tooth Crown Morphology and Testosterone Transfer in Female Members of Opposite-Sex Dizygotic Twin Pairs

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Tooth Crown Morphology and Testosterone Transfer in Female Members of Opposite-Sex Dizygotic Twin Pairs

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Abstract

The twin testosterone transfer (TTT) hypothesis posits that females gestated with male co-twins develop more masculine phenotypes due to in-utero androgen exposure. Research has shown co-gestational effects to be associated with increased deciduous and permanent tooth size in females belonging to opposite-sex dizygotic twin pairs (OSF) as compared with females belonging to same-sex monozygotic (MZF) and dizygotic (SSF) twin pairs and female siblings. This study evaluates whether the TTT hypothesis explains patterns of dental morphological variation, namely differences between OSF and other females (SSF, MZF, female siblings) in a contemporary sample that includes both deciduous and permanent data. This work probes the underpinnings of crown morphology expression, which is assumed to be sexually monomorphic (i.e., male/female data pooled) in applied anthropological research. Resampling statistics and Mann-Whitney U tests were used to compare crown morphology scored using the Arizona State University Dental Anthropology System (ASUDAS), with a focus on canine and molar traits. In the deciduous dentition, significant overexpression in OSF was found for maxillary second molar (m^2) Carabelli's trait, mandibular second molar (m_2) cusp number, and mandibular second molar (m_2) cusp 6, even with application of a Bonferroni correction to account for potential Type I error. In the permanent dentition, highly significant differences in expression between OSF and all other females were noted for two first molar (M1) traits (M^1 hypocone and M_1 cusp 7), even after application of a Bonferroni correction. Again, OSF, on average, showed elevated expression for these traits. These results suggest in-utero hormone exposure leads to elevated expression for some but not all canine and molar traits; the inconsistent results may be due to varying levels of testosterone exposure at critical times during morphogenesis. As such, this thesis lends partial support to the TTT hypothesis. Of note, only a few traits showed strong

overexpression in males across this population, not all of which differed between OSF and other females. This suggests that Y-chromosome effects may be more important than androgen exposure in driving population-level sexual dimorphism in crown morphology.

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Introduction

The twin testosterone transfer (TTT) hypothesis posits that females gestated with male co-twins develop "masculinized" phenotypes due to in-utero testosterone exposure. Previous work has shown an association between hormone and co-gestational effects and increased tooth size in females belonging to opposite-sex dizygotic twin pairs (OSF: male/female cotwins) as compared with females belonging to same-sex monozygotic (MZF: female/female "identical" twins) and same sex dizygotic (SSF: female/female "fraternal" twins) twin pairs (Dempsey et al., 1999; Ribeiro et al., 2013). This study provides yet another test of the TTT hypothesis within the complex system of the dentition. Specifically, this study asks whether twin testosterone transfer explains patterns of variation in crown morphology in a contemporary clinical sample of OS females, SS females, MZ females, and female full siblings. This work probes the non-genetic underpinnings of crown morphology phenotypes, which are generally assumed to be sexually monomorphic in applied dental anthropological research (i.e., male, and female data are often pooled in population-level analyses) (Scott & Turner, 1988; Turner et al., 1991; Scott & Irish, 2013; Irish et al., 2020; Pilloud & Scott, 2020).

Literature Review

Gender and Sex

The terms gender and sex are commonly used interchangeably despite fundamental differences in their formal definitions. Biological anthropologists often deal with biological sex variation, which may or may not correspond to social identity, whether referring to how one self-identifies or how others in the society describe them (Garofalo & Garvin, 2020). "Sex" is generally defined as: (1) either of the two major forms of individuals that occur in many species and that are distinguished respectively as female or male, especially based on their reproductive

organs and structures, or (2) the sum of the structural, functional, and behavioral characteristics of organisms that are involved in reproduction marked by the union of gametes and that distinguish males and females (Merriam-Webster, 2021). The definition implies there are only two categories of sex— female and male— and that classification is based on reproductive organs and gametes. The biology of sex, however, is not as binary as the male/female typology; the development of biological sex is complex and variable. Many individuals are born with variation in elements that define biological sex— from chromosomal arrangements to genital morphology (Blackless et al., 2000). At least 1:1000 individuals have some variation in chromosomal, hormonal, gonadal, or anatomical development that influences sex development and development of secondary sex characteristics, resulting in deviation from these binary categories. For example, humans, are often born with a set of two X chromosomes (XX) or an X and Y chromosome (XY). XX individuals tend to develop female reproductive organs, while XY individuals tend to develop male reproductive organs. Still, individuals may have other chromosomal variants (e.g., XXX, XXY, XO) or experience differences of sexual development (e.g., Complete Androgen Insensitivity Syndrome) that result in biological outcomes that may not fit neatly into two— male and female—sex categories (Ostrer, 2014). In this study, the terms "male" and "female" refer to individuals' sex categories clinically assigned at birth (Bamberger & Farrow, 2021).

Gender is associated with social identities, roles, and constructs and is, in many cases, not biologically determined. Gender identity does not always match expectations associated with assigned biological sex. Gender expression is a persons' external representation of their gender through social and cultural cues (i.e., clothing, hairstyle, speech, and behavior), as well as physical appearance for (e.g., binding, electrolysis, hormone therapy) (Butler, 1990, 1993). In

this sense, gender is performed and constructed by the individual (Butler, 1990). One's gender expression may or may not correspond with their gender identity. Gender identity, instead, corresponds to an internal sense of gender, which can include man, woman, a gender outside of these categories, or no gender (Bamberger & Farrow, 2021).

In this paper, terms such as "masculine" and "feminine" may be used in reference to morphological traits, physiological traits, and behavioral traits. These terms refer to where a trait falls along a spectrum of population variation, in which those categorized as biological males represent one end of the spectrum and biological females represent the other—although I acknowledge this is a simplified representation of sexual variation. Morphological traits that have been previously examined within the twin testosterone transfer (TTT) framework include increased ano-genital distance in animal studies (Ryan and Vandenbergh, 2002) and finger-length ratios where the ring finger is longer than the index finger; both imply increased testosterone exposure in-utero. Physiological traits used to evaluate the TTT hypothesis include auditory system functioning in spontaneous otoacoustic emissions (SOAEs). In humans, females generally exhibit more SOAEs than males, a sex difference that exists from birth (McFadden, 1993). Interestingly, SOAE frequency is lower for OSF than for SSF. Behavioral traits used to test the TTT hypothesis include visuo-spatial response (Vuoksimaaet al. 2010), sensation seeking/aggression (Resnick et al.,1993; Slutske et al., 2011), and sex-typed childhood play (Henderson and Berenbaum, 1997; Rodgers et al., 1998). Note that I use the referenced authors terminology for these characters.

Sex and Development

The endocrine secretion of the gonads determines phenotypic sex and the expression of secondary sex characteristics (Leon, 2016). If male sex hormones and the appropriate receptors

are present, the male genital phenotype will typically develop. Testosterone, produced by the fetal testes, dihydrotestosterone, a metabolite of testosterone, and antimüllerian hormone, determine normal male sexual differentiation, which begins during the seventh week of fetal development (Bakker, 2021). If sufficient male sex hormones or functioning androgen receptors are absent, the female genital phenotype typically develops (Bakker, 2021). Genetically male and female fetuses have undifferentiated gonads during early development; there is no difference in their reproductive structures. Around week six of gestation, the Sry gene on the short-arm region of the Y chromosome initiates testicular differentiation in males (Larney et al., 2014). Development of male phenotype requires a functional Y-chromosome. The absence of a Y-chromosome typically results in the formation of an ovary (Persaud, 2000).

The Leydig cells of the testes are capable of testosterone synthesis by the end of week eight in-utero (Knickmeyer & Baron-Cohen, 2006). Fetal testes begin to produce testosterone prenatally, but the ovaries do not (Wilson et al., 1981). In the female, differentiation of the ovaries begins around week seven of gestation. The fetal ovary is generally considered inactive until late in development (Milmed et al., 2015) but may produce a small amount of estrogen. The amount of estrogen is likely to be insignificant compared with placental estrogen synthesis, and ovarian estrogen production does not seem to have significant effects on sex development until the time of puberty (Fowler et al., 2011). A singleton female fetus is also exposed to low levels of androgens, although a small proportion may come from the fetal adrenals (a biproduct of corticosteroid production) while some arises from the maternal adrenals, ovaries, and fat (Martin, 1985). Testosterone amniotic levels in males are highest from eight to 24 weeks of gestation, with peak levels occurring between 12–18 gestational weeks (Abramovich, 1974; Warne et al., 1977; Nagamani et al., 1979), a time of both rapid brain development and odontogenesis (dental

development). Androgens play a significant role in development influencing both physical and behavioral characteristics (Hines, 2011).

Sexual Dimorphism and Testosterone Transfer

Sexual dimorphism can be defined as phenotypic or observable differences between males and females of the same biological species (Frayer, & Wolpoff, 1985). Dimorphism has been found throughout the human dentition, with males having, on average, larger teeth than females based on crown or cervical mesiodistal and buccolingual measurements of the permanent dentition (Garnet al., 1967; Stroud, et al., 1994; Kondo et al., 2005; Al-Khateeb & Cardoso, 2008; Zorba et al., 2011; Al-Gunaid et al., 2012). Canines are the most sexually dimorphic teeth, with male canines being 5–10% larger than those of females (Hillson, 1996, 2005; Schmidt, 2016). Sexual dimorphism has also been reported for the deciduous dentition, with differences smaller in degree than those reported for the permanent dentition (Harila et al., 2003; Kondo & Townsend, 2004; Anderson, 2005; Harris & Lease, 2005; Adler & Donlon, 2010; Ribeiro et al., 2013). Dental tissue volumes also demonstrate a dimorphic pattern, with enamel crown measurements greater in females and dentine greater in males (Saunders et al., 2007). Measurements obtained by thin sectioning demonstrate that the enamel is responsible for sex differences in the deciduous crown, not the dentine (Saunders et al., 2007). Dentine and root volumes are greater in males as measured by micro-CT (Fernée et al., 2021).

In comparison with metric phenotypes, it is commonly reported that dental morphological traits are characterized by limited sexual dimorphism (Scott & Turner, 1988;Turner et al., 1991; Scott & Irish, 2013; Irish et al., 2020). Low levels of dimorphism have been reported for crown morphology, with only one trait (canine distal accessory ridge) showing consistent differences between males and females (Pilloud & Scott, 2020). For this reason, male and female crown and

root morphology data are often pooled to estimate population frequencies in biodistance analyses and studies of microevolutionary processes (Irish et al., 2020; Pilloud & Scott, 2020). Carabelli's trait expression is sexually dimorphic in some populations; in these samples, males often exhibit high degrees of expression (the cuspal form), while females exhibit low degrees of expression (absence or groove form) (Kieser, 1984; Hsu et al., 1997; Kondo & Townsend, 2006). Varying degrees of dimorphism have been noted for deciduous morphology. Some traits show no dimorphism (Joshi et al., 1972; Kieser, 1984; Hsu et al., 1997), while dimorphism has been reported for some traits observed in a European Australian sample (Taduran, 2018). However, limited research has focused on the biological underpinnings of sexual dimorphism in human crown morphology.

The twin testosterone transfer (TTT) hypothesis posits that human sex hormones are transferred between multiple-birth siblings or co-twins in-utero impacting ultimate phenotypic trait expression. Transfer of testosterone from male co-twin to female co-twin may occur via a) maternal circulation, or b) directly between fetuses (Miller, 1994). Empirical evidence for the maternal circulation pathway comes from animal studies showing that testosterone injected into a pregnant mother increases circulating testosterone concentrations in the gestating fetus and exerts a masculinizing influence on offsprings' postnatal behavior and anatomy (Phoenix et al., 1959; Miller, 1994), including increased ano-genital distance (Lephart et al., 1989), tendency toward infanticide, and elevated levels of rough-and-tumble play—a behavior more common in juvenile males than in juvenile females (vom Saal, 1983; Hines, 2006). In humans, hormone transfer to fetus via the maternal circulation route is not supported, because hormone levels in maternal blood and amniotic fluid do not appear to be correlated (Nagamani et al., 1979). Human studies have also found that fetal sex cannot be predicted from maternal serum androgen

concentrations (Glass & Klein, 1981; Hines et al., 2002; van de Beek et al., 2004), which suggests maternal-fetal hormone transfer is unidirectional, with hormones passing solely from the mother to the fetus (Tapp et al., 2011). The term "androgens" includes substances, including testosterone, 0 promote masculinization. Androgens are produced by the testes, adrenal glands, and ovaries, but the testes are the primary source (Hines, 2011). The second potential route for testosterone transfer runs directly between fetuses (diffusion across fetal membranes). Amniotic fluid can permeate the fetal skin and the placenta until week 18 of gestation when testosterone production in males is at its peak (Abramovich & Page, 1972; Abramovich, 1974; Nagamani et al., 1979). This suggest that females may be exposed to elevated levels of testosterone in-utero due to the presence of their male co-twins.

An ideal measure of prenatal hormone exposure would be serial sampling of hormone levels at distinct points throughout gestation, infancy, childhood, and adulthood. Unfortunately, because there is risk associated with the collection of serum from a fetus, this approach is not feasible (Cohen-Bendahan et al., 2005b). Surrogate measures have included androgen concentrations in maternal serum during pregnancy (Hickey et al., 2009) and perinatal hormones obtained from umbilical cord blood at birth (Whitehouse et al., 2010; Galiano et al., 2021). Maternal plasma testosterone shows no significant differences between those gestating male versus female offspring. The maternal compartment does not seem to contribute significantly to the steroids in amniotic fluid, and there is no correlation between maternal serum and amniotic fluid levels (Nagamani et al., 1979). It has also been suggested that studies of prenatal hormones sampled from the amniotic sac during the second trimester of pregnancy provide the most accurate measure of fetal androgen exposure (Cohen-Bendahan et al., 2005b), but this procedure

is only performed out of medical necessity, and the participant sample may not be representative of the general population (Tapp et al., 2011).

Studies combining maternal serum, amniotic fluid, and fetal serum levels are limited. Rodeck and colleagues, in pursuit of rapid fetal sex prediction by maternal serum or amniotic fluid, combined measurements of the three compartments: maternal serum, amniotic fluid, and fetal serum. There was too much overlap in male-female hormone ranges for these measures to be useful for biological sex estimation (Rodeck et al.,1985). Notwithstanding, the findings are useful for understanding relative androgen levels, which is essential for contextualizing reported behavioral and morphological variation for opposite-sex twin females (OSF). Amniotic fluid testosterone levels were significantly higher in fetal males than fetal females, with the average magnitude of difference being 1.18 nmol/l in males versus 0.73 nmol/l in females. Fetal plasma testosterone levels were 5.01 nmol/l in males versus 1.02 nmol/l in females (Rodeck et al.,1985). These results corroborate earlier reported maternal plasma testosterone levels (Warne et al., 1977; Nagamani et al., 1979; Glass & Klein, 1981). Fetal plasma testosterone levels agree with those reported in earlier studies, as well (Reyes et al., 1974; Abramovitch et al., 1978). To date, there has been no measurement of testosterone levels in OS twin pairs' amniotic fluid, let alone prenatal fetal serum measures. Studies like Rodeck et al. (1985) are unlikely to be repeated, because percutaneous fetal blood sampling carries a 1.4% risk of fetal loss and, therefore, has fallen out of favor in the medical research community (Van Kamp et al., 2005). Instead, phenotypes in female-male twin pairs provide a natural experiment to test whether proposed hormone diffusion occurs in-utero as outlined by the TTT hypothesis and to explore the potential effects of this phenomenon (Dempsey et al., 1999).

Differences in androgen levels and hormone effects arise from the distinct functional strategies of the ovaries and testes. In males, prenatal testes produce testosterone to maintain the germ cells in a premeiotic stage (Carlson, 2018). Prenatal ovaries generate a full complement of ova, which have already entered the initial stages of meiosis. Prenatal ovaries are endocrinologically inactive, with granulosa cells only beginning to function after birth (Carlson, 2018). For this reason, any hormonal effects associated with opposite-sex twin co-gestation is expected to be more pronounced in female than in male twins (Tapp et al., 2011), because females produce little estrogen and testosterone.

Animal Studies

Initial studies of prenatal hormone effects centered around non-human animals. In an early study, Phoenix, and colleagues (1959) found that female guinea pigs prenatally exposed to testosterone showed masculinized behavior (mounting behavior) in adulthood (Phoenix et al., 1959). Since then, numerous animal studies have demonstrated testosterone effects on neurobehavioral sexual differentiation (Constantinescu & Hines, 2012). The phenotypic effects of prenatal androgen exposure have been investigated within an experimental framework by administering varying doses of testosterone in-utero. A dose–response relationship has been observed, in which fetuses exposed to higher doses of testosterone experience greater phenotypic change than fetuses exposed to lower doses (Wolf et al., 2002; Hotchkiss et al., 2007). Hormone concentrations necessary to alter trait expression vary across phenotypes (Cohen-Bendahan et al., 2005b). In litter-bearing mammals, gestating offspring may be subjected to differing hormonal environments based upon the sex of neighboring fetuses. Murine models have demonstrated that sex hormone exposure is influenced by the intrauterine positioning of the animal (vom Saal & Bronson, 1980; vom Saal et al.,1990). Female rats and mice are more sensitive to intrauterine

position effects than males (Ryan & Vandenbergh, 2002). A fetus located between two developing males has higher blood concentrations of testosterone and lower blood concentrations of estradiol than a fetus located between two developing females (vom Saal, 1989; Ryan & Vandenbergh, 2002). Further, female rodents developing between males in-utero express masculinized anatomical (increased anogenital distance), behavioral (more aggressive behavior, less attractive to males), and reproductive characteristics (less reproductive success) in adulthood compared with females that develop near other females in-utero (Ryan & Vandenbergh, 2002). Some investigators, however, have cautioned this phenomenon may not apply to species with distinct life histories (Fishman et al., 2019), and, as such, the degree to which these results apply to humans is unclear (Bracken, 2009).

Congenital Adrenal Hyperplasia

Congenital adrenal hyperplasia (CAH) provides a framework for exploring the influence of testosterone on human neurobehavioral development (Constantinescu & Hines, 2012). Individuals with CAH produce elevated levels of androgens from early in gestation, due to an enzymatic defect caused by a single gene: 21-hydroxylase deficiency (>90% of cases) (Pang et al., 1980; White, 2009). Amniotic fluid testosterone in females with CAH is elevated early and throughout gestation with levels equal to or exceeding those of a normal male fetus (Carson et al., 1982; Wudy et al.,1999). The overproduction of androgens during fetal development causes virilization of the external female genitalia ranging from mild clitoral enlargement to complete fusion of the labioscrotal folds with a phallic urethra (Acién & Acién, 2020). Females with CAH are suggested to differ from unaffected females in several behavioral domains, including activity interests, personality, cognitive abilities, handedness, and sexuality (Hines et al., 2003). For example, studies of childhood play by CAH females (using the authors' terms) have shown

increased male-typical toy, playmate, and activity preferences (Hines, 2011). Young CAH females exhibit low interest in dolls and, later in life, little interest in child rearing. CAH girls may be "tomboyish" regarding their dress and lack of interest in make-up and jewelry (Hines, 2011). They demonstrate more aggressive behavior than their peers and male-typical behavior in social relations (using the authors' terms). Male-typical cognitive traits including heightened spatial abilities and increased frequency of left handedness have been reported in females with CAH (Hall et al., 2004). Males with CAH are like their unaffected male siblings with respect to most aspects of behavior (Hampson et al., 1998; Hines et al., 2003).

Studies of CAH females have three limitations. First, CAH females have high androgen levels, and it is unclear how within-sex variation in hormones affects behavior. Second, high androgen levels may persist postnatally, so it is difficult to separate in-utero versus postnatal hormonal effects. Third, the preferences and behaviors of CAH females may be impacted by differential treatment in response to their expression of masculinized primary and secondary sex characteristics (Quadagnoet al., 1977). It can be reasonably argued that, in humans, sex assignment at birth influences parental attitudes toward the infant and that these social factors are essential in determining gendered behavior of the infant while hormones play only a minor role. Studies dealing with the dental correlates of CAH are limited to case reports of premature exfoliation of primary teeth and accelerated eruption of permanent teeth. In one case report, root development of the permanent mandibular central incisors and eruption of the permanent first molars at the age of 4.5 years suggest dental age can be advanced in females with CAH (Singeret al., 2000; Angelopoulouet al., 2015).

Twin Models

Twins have been used in numerous studies to address questions relating to genetic, environmental, and epigenetic contributions to phenotypic variation in humans. They provide a unique experimental "system," in which gestational effects are held constant and, in the case of monozygotic twins, underlying genotypes are (theoretically) identical (Schwab & Hogenson, 2017; Hall, 2021). Twins are not rare: the spontaneous rate of twinning is about 1 in 80 livebirths, which means that about 1 in 40 individuals is a twin (Hall, 2003). The monozygotic twinning rate is lower (0.4% of births) and constant around the world (Derom et al., 1987; Satio et al., 2000). Any reported regional disparities in twining rates represent variation in dizygotic twinning (Nylander, 1975).

Twin models are used extensively to study the genetic basis of complex traits and diseases (Boomsma et al., 2002; Townsend et al., 2012). Opposite sex dizygotic twins (i.e., male/female fraternal twins) are studied to discern the effects of hormones and sex chromosomes on phenotype expression, for example dental morphological traits. Dizygotic twins (DZ) share, on average, 50% of their genes (Falconer & Mackay,1989) and arise from two separately fertilized eggs. They are expected to differ in their genetic and environmental profiles. In contrast, monozygotic twins (MZ) result from fertilization of a single egg that separates into two zygotes that are expected to share a fully identical genetic profile (Townsend et al.,2005; Race et al., 2006; Townsend et al., 2009; Balasuramanian, et al., 2012; Hall, 2021). Comparison of MZ and DZ twins provides an opportunity to explore whether phenotypic characters of interest are more strongly influenced by genetic or environmental factors. Comparison of DZ same-sex pairs (SS) and DZ opposite-sex pairs (OS) provides an opportunity to investigate whether primary or secondary sex characteristics are affected by sex-dependent environmental factors, including

chromosomal and hormonal effects (Voracek & Dressler 2007; Ribeiro et al., 2013; Benetos et al., 2014).

A significant body of research has explored intrauterine effects and their phenotypic correlates in human twin pairs. Several review articles have summarized the findings of sixty papers published through 2020. Miller (1994) concluded that comparison of OSF and SSF twins provides a reasonable model for prenatal testosterone exposure. Subsequently, Cohen-Bendahan and colleagues' (2005b) review focused on the effects of prenatal sex hormones on sex-typed/gendered behaviors and concluded there is solid evidence that behavioral patterns are influenced by sex hormones during prenatal development. However, a more recent metanalysis of human studies of OSF and SSF twins found there is inconsistent support for the TTT hypothesis (Tapp et al., 2011). Ahrenfeldt (2020) also found a limited number of differences in physiological, cognitive, and behavioral disparities between OS and SS females.

Because cognitive and behavioral traits are difficult to quantify, the dentition may be a more appropriate system for evaluating the TTT hypothesis. This is, in part, because tooth form is determined early in development and does not remodel or change throughout the life course unless acted upon by outside mechanical processes (e.g., wear, erosion, trauma). The process of odontogenesis in humans begins at around six weeks in utero and extends until the late teenage years, when the roots of the third molar teeth are formed (Massler et al., 1941; AlQahtani et al., 2010). Ectodermal and ectomesenchymal tissues regulate the process of odontogenesis, including initiation, morphogenesis, and differentiation. Initiation starts with the thickening of the oral epithelium at specific sites to form dental placodes and determines tooth region and number. Morphogenesis includes epithelial-mesenchymal reciprocal signaling (Mass & Bei, 1997) with proteins acting as either activators or inhibitors at specific places and times that result in enamel

knots formation at the site of future cusp tips. This, coupled with the folding of dental epithelium, result in distinct tooth shape, size, and cusp number in species with multicupid teeth, including humans (Jernvall & Thesleff, 2000; Guatelli-Steinberg et al., 2013). This is followed by histodifferentiation of ameloblasts and odontoblasts, which secrete dental hard tissues: enamel and dentin, respectively (Brook et al., 2014a; Nanci, 2017). The eventual tooth phenotype results from the interplay of genetic, environmental, and epigenetic inputs (Brook et al., 2014a; Townsend et al., 2015).

Prenatal testosterone has a potential epigenetic effect on dental morphology (Miller, 1994; Dempsey et al., 1999; Ribeiro et al., 2013; Taduran et al., 2018). There are three surges of testosterone that occur in normal male development. The first surge begins at around the seventh to ninth week of pregnancy (following testicular differentiation), and testosterone levels peak between 12–18 gestational weeks (Abramovich, 1974; Reyes et al., 1974; Warne et al., 1977; Nagamani et al., 1979; Knickmeyer & Baron-Cohen, 2006). The second surge occurs after birth with separation from placental estrogen (Griffin & Wilson, 2003). The third surge occurs during puberty. Timing of testosterone elevation and tooth morphogenesis is summarized in Figure 1.

Initial stages of dental development involve epithelial structures that are malleable or susceptible to environmental and epigenetic effects. Later in odontogenesis, hard tissue secretion and calcification preserves ultimate tooth morphology (Jernvall & Thesleff, 2000; Brook, 2009; Guatelli-Steinberg et al., 2013; Brook et al., 2014a; Nanci, 2017). Primary (deciduous) and secondary (permanent) dentitions form at separate times, exposing developing teeth to differing levels of hormonal effects (Fig. 1). Importantly, the primary tooth crowns, as well as some permanent tooth crowns, begin formation in-utero, which means they are exposed to gestational hormonal effects. The deciduous teeth begin development around 4 to 6 weeks post-conception

and continue crown development until around one year after birth (Lunt & Law, 1974; Irurita et al., 2014). The permanent teeth start to form at approximately 14 weeks post-conception and continue formation through third molar crown calcification, completing at approximately 14 years of age (AlQahtani et al., 2010). As such, the primary dentition develops over a shorter period and, consequently, has less testosterone exposure in terms of time but greater exposure in terms of in-utero concentration. If we map dental development onto hormonal levels (Fig.1), we see that, by the time male gestational testosterone levels have surged, peaked, and leveled, primary teeth have already passed through all soft tissue stages of tooth formation before enamel calcification (Abramovich, 1974; Reyes et al., 1974; Warne et al., 1977; Nagamani et al., 1979; Knickmeyer & Baron-Cohen, 2006). Testosterone levels remain fairly stable throughout the remainder of gestation at a considerably lower-level (Nanci, 2017) (Fig. 1).

The epigenetic effect of testosterone has been studied by exploring differences in genome-wide DNA methylation and histone modification between OSF and SSF twins (Kong et al., 2020). Human germline cells undergo overall DNA demethylation from seven to 19 weeks, corresponding closely with testosterone production (Wen & Tang, 2019). Therefore, epigenetic modification may play a specific role in TTT and its effects on dental phenotypes in opposite-sex twins. Based on DNA methylome data, OSF have different epigenetic markers compared to SSF; DNA methylation and histone modification data show greater correspondence between OSF and OSM/SSM than between SSF and males (Wen & Tang, 2019; Kong, et al., 2020). These data suggest “masculinization” of the OSF epigenome. In contrast, OSM and SSM did not significantly differ, which indicates OSM may be less epigenetically influenced by the presence of female co-twins in-utero (Kong, et al., 2020). In one study, DNA methylome changes were associated with nervous system development and regulation of neural crest derived structures

during a period in which tooth formation occurs (Ornoy, 2020). Still, the extent to which in-utero hormonal effects impact ultimate crown morphology of the deciduous and permanent teeth remains unclear.

Research Aims and Hypothesis

The overarching goal of this research is to explore the effects of intrauterine hormone exposure on dental morphological phenotypes. Here, I examine the phenomenon of twin testosterone transfer (TTT) using tooth crown data collected from a longitudinal human sample representing dizygotic opposite sex twin females (OSF), same-sex dizygotic twin females (SSF), monozygotic twin females (MZF), and sister siblings. My null hypothesis states that in-utero hormone diffusion from male to female co-twin has no significant impact on dental morphological phenotypes. Under this hypothesis, I expect to find no morphological differences between OSF and SSF, MZF, and female siblings in the deciduous or permanent dentition.

Materials and Methods

The data used in this study represent participants of the University of Adelaide Twin Study (UAT) Cohort 2. Nearly all participants are of European ancestry and are residents of the metropolitan regions of Adelaide, South Australia and Melbourne, Victoria (Townsend et al., 2015). UAT Cohort 2 twins' zygosities were initially confirmed by analyses of up to six highly variable genetic loci on six different chromosomes using DNA obtained from buccal smears (Hughes et al., 2013). This study used an existing morphological dataset collected from anonymized dental models as part of a previous study (Paul, 2017; Paul et al., 2020). Crown morphology was recorded following Arizona State University Dental Anthropology System (ASUDAS) standards originally described by Turner et al. (1991). The data were sorted by

documented sex, zygosity, and includes siblings. Initial data collection and the application of the recorded, anonymized dataset to this study were approved by the University of Adelaide Human Research Ethics Committee Approval—Project H-27-1990. This project was also deemed exempt from review by University of Arkansas' Institution Review Board (IRB) by the 2020 IRB coordinator.

Dental morphology is generally assumed to be sexually monomorphic. Dimorphism, when reported, tends to be limited to canine and molar crown traits (Moreno-Gómez, 2013; Pilloud & Scott, 2020). For this reason, I have limited my test of the TTT hypothesis to crown characters of the canines and molars. Data for 17 deciduous characters were included in the study. The deciduous sample represents 12 dizygotic opposite-sex twin females, 31 dizygotic same-sex twin females, 70 monozygotic twin females, and 77 female siblings. Data for 27 permanent characters were included in the study. The permanent sample represents the same 12 dizygotic opposite-sex twin females and 31 dizygotic same-sex twin females, 67 monozygotic twin females, and 77 female siblings. Males were evaluated for the same 17 deciduous and 27 permanent traits to assess overall levels of sexual dimorphism in the population (deciduous dataset: 12 dizygotic opposite-sex twin males, 34 dizygotic same-sex twin males, 44 monozygotic twin males, and 39 male siblings; permanent dataset: 12 dizygotic opposite-sex twin males, 32 dizygotic same-sex twin males, 40 monozygotic twin males, and 60 male siblings).

For each trait, the highest degree of expression across an individual's left and right antimeres was preserved as the "maximum expression" score. When data for either the left or right side was missing, the sole available score was included in the dataset. These steps are commonplace in dental morphology studies; they mitigate genetic redundancy in the data set

given the strong correlation between morphology scores across antimeres (Turner & Scott, 1977; Turner et al., 1991) and provide the largest possible sample size for the study (Paul et al., 2021). Table 1 lists morphological traits, abbreviations, teeth scored, and ASUDAS grade ranges for each trait.

The ASUDAS standardizes nonmetric dental morphological features visible on crown surfaces of teeth (Scott & Turner, 1988; Turner et al., 1991). Some traits, such as cusp number, provide count data. Other traits, for example, distosagittal ridge, record the presence or absence of a trait. However, for most dental nonmetric traits, such as incisor shoveling and Carabelli's trait, expression is recorded as ordinal data that describe ranked values (i.e., small to large). This system allows researchers to observe range of expression, reduce error, and provide comparable datasets (Turner et al., 1991; Buikstra & Ubelaker, 1994).

To compare trait expression between OSF and all other females in the sample, I used non-parametric Mann-Whitney U tests (Corder & Foreman, 2014). In the Mann-Whitney U, two independent samples are combined and rank-ordered to determine if the values from the OSF and broader female sample are randomly mixed in the rank ordering or clustered at opposite ends of the distribution. This test makes two assumptions: 1) the underlying level of measurement is continuous, although the units of observations are discrete (crown morphology data are generally assumed to be characterized by an underlying, continuous distribution), and 2) the samples are independent (Madrigal, 2012).

Due to the relatively limited OSF sample size, significance testing was based on random resampling of the Mann-Whitney U via Monte Carlo method set to 10,000 iterations. To account for potential Type I error associated with multiple hypothesis testing, I applied a conservative

Bonferroni correction ($\alpha = \alpha'/n$). For example, significance for each of the 17 deciduous trait models was evaluated with reference to an adjusted α of 0.003 (or 0.05/17).

Both deciduous and permanent dizygotic female/male twin data sets were compared using Mann-Whitney U tests and are presented in tables four and five. A Wilcoxon signed-rank test was not used for within-twin pair comparisons, because missing data varied across traits which would lead to omission of several matched pairs for comparison. Additionally, the entire sample of UAT Cohort 2 females and males were compared using Mann-Whitney U tests to assess overall levels of sexual dimorphism for any traits flagged for potential TTT effects (i.e., traits that differed in expression between OSF and all other females). Statistical analyses were calculated using SPSS Statistics for Windows (Version 27.0) and XLSTAT (Addinsoft, 2021).

RESULTS

Deciduous Morphology

Table 2 presents summary statistics for the deciduous dental traits and compare dizygotic OSF and all other females in the sample (DZFs, MZFs, female siblings). Mann-Whitney U comparison of OSF and all other females indicated significant difference for three traits with the conservative application of Bonferroni correction ($p < 0.003$): m^2 Carabelli's trait, m_2 cusp number, and m_2 cusp 6. Three other traits' expression significantly differed between the two groups without application of Bonferroni correction ($p<0.05$): c^1 shoveling, c^1 tuberculum dentale, and m_2 deflecting wrinkle. The arithmetic means for each of these six statistically significant traits were greater for the OSF than all other females in the sample.

Deciduous c^1 shoveling (OSF median = 1.5, other F median = 1.0; OSF mean = 1.3, other F mean = 0.8) and c^1 tuberculum dentale (OSF median = 1.0, other F median = 1.0; OSF mean =

2.9, other F mean = 1.7) were minimally expressed in the study groups, but expression was significantly higher in OSF versus all other females (Figs. 2-3). Figure 4 illustrates m^2 Carabelli trait's strong expression in this sample, a pattern typical of many populations of European ancestry (OSF median = 6.0, other F median = 4.0; OSF mean = 5.8, other F mean = 4.4) (Scott, 1980; Lee & Scott, 2011). Again, the OSF significantly overexpressed this trait compared to the SSF, MZF, and sister siblings. The m_2 deflecting wrinkle (OSF median = 2.0, other F median = 1.0; OSF mean = 1.6, other F mean = 1.1) and m_2 cusp number (OSF median = 5.0, other F median = 5.0; OSF mean = 5.4, other F mean = 5.1) showed intermediate expression. Expression was minimal for the other significant m_2 character, m_2 C6 (OSF median = 0.0, other F median = 0.0; OSF mean=0.6, other F mean = 0.2). The OSF again overexpressed these traits (Figs. 5-7).

Permanent Morphology

Table 3 presents the summary statistics for the permanent dental traits and compares OSF and all other females in the sample. Mann-Whitney U comparisons indicated significant difference in expression between OSF and all other females for two traits with the conservative application of Bonferroni correction ($p < 0.002$): M^1 hypocone and M_1 cusp 7. Three other traits achieved statistical significance at $p<0.05$: C^1 double shoveling, M_1 cusp 5, and M^1 Carabelli trait. Figure 8 shows moderate C^1 double shovel expression (OSF median= 3.0, other F median = 2.0; OSF mean= 3.0, other F mean = 2.3) with statistically significant overexpression in the OSF. The M^1 hypocone results are shown in Figure 9. This trait is strongly expressed in both comparison groups, with significant overexpression in OSF (OSF median = 5.5, other F median = 5.0; OSF mean=5.5, other F mean = 5.0). M^1 Carabelli trait is strongly expressed in OSF (median=6.0, mean=5.7) and moderately expressed in all other females (median = 5.0, mean = 4.2). This is graphically represented in Figure 10. M_1 C5 is moderately expressed in both groups,

with overexpression in OSF (OSF median = 4.0, other F median = 4.0; OSF mean= 3.9, other F mean = 3.2).

Overall Sexual Dimorphism

For traits found to be significantly elevated in expression between OSF and all other females, I tested for sexual dimorphism in the overall sample. All females were compared to males in UAT Cohort 2 using Mann-Whitney U tests. Of the deciduous traits flagged for potential TTT effects (Table 4), c^1 shoveling is significantly overexpressed in males (males: median = 1.0, mean = 1.1; females: median = 1.0, mean = 0.9; p = 0.003, Bonferroni-corrected α= 0.003). Interestingly, m^2 cusp 5 is over-expressed in females (males: median = 0.0, mean = 0.2; females: median = 0.0, mean = 0.3; p = 0.02), although this result was not significant with application of a conservative Bonferroni correction. Beyond these two traits, no sexual dimorphism was detected in deciduous morphology in UAT Cohort 2. Of the permanent traits flagged for potential TTT effects (Table 5), males are characterized by stronger distal accessory ridge expression in both canines—significantly so for the maxillary canines (C^1 males: median = 3.0, mean = 2.9; C^1 females: median = 2.0, mean = 2.1; p<0.0001, Bonferroni corrected α = 0.002). Males showed slightly elevated expression for C^1 double shoveling, M_1 protostylid, and M_1 C 7 (tuberculum intermedium) (Table 5). Sex differences for these traits were not statistically significant with the application of conservative Bonferroni correction.

OS Twin Pairs

I also compared female-male trait expression for the sample of OS twin pairs (maximum n = 12). Table 6 presents a comparison of deciduous trait expression in OSF and OSM. No statistically significant difference was found for female-male dizygotic twin trait expression. Table 7 presents a comparison of permanent trait expression for the sample of OSF and the sample of OSM. Values differed only for C^1 distal accessory ridge ($p = 0.005$, Bonferroni-corrected $\alpha = 0.002$). Here, I found stronger expression in males (median = 4, mean = 3.4) than in females (median = 0, mean = 0.6).

Post-hoc evaluation shows no pattern of individual OSF or OSM driving overexpression (Tables S1-S2). When comparing canine to molars in the OS twin pairs, deciduous morphology is comparable in the females and males (Table S3), however, permanent molars show a trend toward overexpression in males (Table S4). These results (that a pair or set of pairs are not driving the results of the TTT study) are not surprising, as crown morphology is not like crown size in that correlation among traits is complex. Morphology manifests as complex combinations of negative (groove, divots) and positive (ridges, crests, cusps) features on the crown surface, and previous studies have shown relatively low genetic and phenotypic correlation among these traits, even those expressed on the same tooth (Stojanowski et al., 2018, 2019; Paul et al., 2021). Therefore, I would not expect a single individual or twin pair to be overexpressed for *all* traits and drive the entirety of the results.

Discussion

In applied studies of dental morphology, male and female data are often pooled, based on the general assumption that crown traits are monomorphic, apart from some canine and molar characters. Recent research provides some support for this assumption at a genetic level, as sex is a significant covariate in few heritability models of trait variation, the exceptions being C^1 and C_1 distal accessory ridge (Paul et al., 2020), M^2 hypocone, M^1 Carabelli's trait, M_2 cusp 6, and M_1 cusp 7 (Stojanowski et al., 2019). It is for this reason that I limited tests of the TTT hypothesis to traits of the canines and molars. Sex differences for specific traits vary (Pilloud & Scott, 2020). For Carabelli's trait, some researchers have reported no difference between males and females (e.g., Garn et al., 1966; Turner, 1969; Bang & Hasund, 1972; Scott, 1980; Townsend, 1992), while others indicate significant sex differences (e.g., Goose & Lee, 1971; Joshi et al., 1972; Kaul & Prakash, 1981; Kieser & Preston, 1981; Townsend & Brown, 1981; Scott et al., 1983; Mizoguchi, 1985). Studies demonstrating dimorphism in M^1 Cusp 5 have shown that greater expression in males may be related to their overall larger teeth (Kondo et al., 1998; Agnihotri & Sikri, 2010; Abrantes et al., 2015). When significant male-female differences are reported for these traits, males typically show higher frequencies and more pronounced expressions (Pilloud & Scott, 2020). This is noteworthy, because in all traits flagged for potential TTT effects in the study, OSF showed elevated trait expression compared to all other females.

The results of this study led to a rejection of my null hypothesis for some but not all traits, as a number of morphological characters significantly differed between OSF and all other females. In the deciduous dentition, significant overexpression in OSF was found for m^2 Carabelli's trait ($p=0.002$), m_2 cusp number ($p=<0.0001$), and m_2 cusp 6 ($p=<0.0001$), even with application of a Bonferroni correction to account for potential Type I error. Second deciduous

molar morphogenesis begins before the sharp uptick of male testosterone production in-utero and extends throughout peak production (gestational weeks 12-18) (Fig. 1; Ribeiro et al., 2013). For this reason, it is not surprising that hormone diffusion from a male-cotwin might impact female trait expression for this tooth. Two deciduous canine traits and an additional molar trait significantly differed in expression between OSF and all other females without application of a Bonferroni correction: c^1 shoveling, c^1 tuberculum dentale, and m_2 deflecting wrinkle trait. Canine morphogenesis begins in-utero before the male testosterone surge, and calcification is completed during the peak in gestational testosterone production (Fig. 1; Ribeiro, et al., 2013). As such, deciduous canines have a shorter exposure to testosterone than do deciduous second molars. This might explain why a weaker TTT effect was detected for canine morphology compared to molar morphology. Interestingly, once again, for all traits flagged for potential TTT effects in the study, OSF showed elevated trait expression compared to all other females.

In the permanent dentition, I found significant differences in expression between OSF and all other females for two M1 traits (M^1 hypocone and M_1 cusp 7) even after the application of a Bonferroni correction. Three other traits achieved a statistical significance of $p<0.05$: C^1 double shoveling, M^1 Carabelli's trait, and M_1 cusp 5. Again, OSF, on average, showed elevated expression for these traits. M1 morphogenesis begins slightly after peak testosterone levels for males in-utero and extends to 37-38 weeks gestation, translating to a prolonged exposure to testosterone in comparison to the permanent canine (Fig.1; Ribeiro, et al., 2013). Carabelli's trait is the one trait for which a TTT effect was detected in both the deciduous and permanent dentitions. This could be accounted for by hormonal or non-hormonal (i.e., genetic) factors. When this trait is present in dm2 it is often associated with expression in M1 (Edgar & Lease 2007), which suggests either a) testosterone effects in-utero have significant effects on both

deciduous and permanent expression for this trait or b) strong pleiotropy for these homologous characters. Given our understanding of the timing of dental development and testosterone exposure (Fig.1; Ribeiro, et al., 2013) and genetic correlation among molar characters for this sample (Paul, 2017), it is likely both genetic and hormonal factors are at play in the consistent overexpression of Carabelli's trait in OSF.

Based on my results, OSF are indistinguishable from OSM in their dental morphological profiles. This is true for the deciduous dentition and permanent dentition, except for c^1 distal accessory ridge, which is overexpressed in OSM. However, when examining levels of sexual dimorphism across the entire sample, two deciduous traits significantly differed in male and female expression (c^1shovel: male > female and m^2 c5: female > male). In the permanent teeth, there was strong overexpression in males for select traits: C^1 double shovel, M_1 protostylid, and M_1 cusp 7. Additionally, difference in male/female expression was indicated for the one morphological trait commonly considered to be sexually dimorphic based on data reported from numerous global samples: distal accessory ridge of the canines (Scott, 1977, Kaul & Prakash, 1981; Kieser & Preston, 1981; Scott et al., 1983; Scott & Turner, 1997; Abrantes et al., 2015). This trait was found to be over-expressed in males in both the maxillary and mandibular dentition. Interestingly however, OSM and OSF expression also differed ($p=0.005$), suggesting that hormones play a limited role in sex-specific canine distal accessory ridge formation. It is possible that sexual dimorphism for this trait might be the result of a growth promoting effect of the Y-chromosome (Alvesalo, 2009). This interpretation is further supported by the fact that we did not detect a TTT effect for canine distal accessory ridge based on comparisons of OSF and all other females.

Alvesalo and colleagues have studied the influence of sex chromosomes on dental development by examining patients with chromosomal aneuploidies. Their findings indicate that both the X- and Y-chromosomes are responsible for crown development, with the Y-chromosome promoting proliferation of both enamel and dentin and the X-chromosome influencing the development of enamel only (Alvesalo et al., 1975; Alvesalo & Kari, 1977; Alvesalo & Varrela, 1980; Alvesalo & Tammisalo, 1981; Alvesalo, 2009). These researchers have proposed that sexual dimorphism in the human dentition is due mainly to the effects of the sex chromosomes. Although sex chromosomes seem to be responsible for some dental variation, other environmental factors, such as sex hormones, should also be considered when assessing sexual dimorphism in tooth morphology, as suggested by the findings of the current study.

This study adds to our knowledge of the effects of sex hormones on the human dentition and provides support for TTT hypothesis by demonstrating that exposure to male hormones in-utero likely has a small but, in some cases, significant impact on both deciduous and permanent trait expression in co-gestated siblings. Documented testosterone secretion surges and timing of individual tooth morphogenesis in-utero correlate well with elevated expression for some characters in OSF compared to other females, namely in the deciduous m2 and permanent M1. This may reflect an epigenetic effect as suggested by Kong et al. (2020), in which DNA methylation of OSF is significantly influenced by the presence of a male co-twin with potential impacts on nervous system development and regulation. This embryologic timing is congruent with odontogenesis (Chai et al., 2000; Hines, 2011; Kong et al., 2020). However, even for some sexually dimorphic traits, hormones seem to have a limited effect. Of note, patterns of canine distal accessory ridge expression in this sample support genetic/Y-chromosome control for

certain characters, underscoring the complex biological foundations of sexual dimorphism in the human dentition.

Conclusions

This study examined dental morphological variation in a European Australian sample within the framework of the TTT hypothesis. The overreaching aim of this work was to improve our understanding of how environmental factors, specifically hormone exposure throughout odontogenesis, influence dental morphology in the human diphyodont dental complex. My findings show some support for the TTT hypothesis. Females from OS twin pairs show statistically significant elevation in expression for some crown characters, as quantified using ASUDAS criterion. Of the traits for which a TTT effect was detected, most involved the deciduous and permanent molars. Differential developmental timing and in-utero testosterone exposure between the canines and molars may account for this result. However, not all traits overexpressed in OSF were found to be sexually dimorphic across the entire sample. Conversely, not all sexually dimorphic traits differed in expression between OSF and other females. This suggests a potential Y-chromosome effect as opposed to environmental androgen-exposure effect for certain characters, as all but one of the sexually dimorphic traits in this sample showed elevated expression in males.

Table 1: Traits, Abbreviations, Elements Scored, and ASUDAS Grade Range

Morphological crown traits for UAT data collection			
Morphological Trait[a]	Abbreviation	Dental Elements Scored	Grade Range[b]
Shovel	SHOV	c^1, C^1	0-7
Double Shovel	DSHOV	c^1, C^1	0-6
Tuberculum Dentale	TD	c^1, C^1	0-7 (includes 5-)
Distal Accessory Ridge	DAR	c^1, C^1, c_1, C_1	0-5
Metacone	META	m^2, M^1, M^2	0-6 (includes 3.5)
Hypocone	HYPO	m^2, M^1, M^2	0-6 (includes 3.5)
Cusp 5 (Metaconule)	C5	m^2, M^1, M^2	0-5
Carabelli's Trait	CTRAIT	m^2, M^1, M^2	0-7
Parastyle	PARA	m^2 M^1, M^2	0-5
Cusp 5	C5	m_2, M_1, M_2	0-5
Cusp 6	C6	m_2, M_1, M_2	0-5
Cusp 7	C7	m_2, M_1, M_2	0-5 (includes 1A)
Cusp Number	CNO	m_2, M_1, M_2	4-6+
Anterior Fovea	ANT FOVEA	m_2, M_1	0-4
Deflecting Wrinkle	DWRINK	m_2, M_1	0-3
Protostylid	PSTYLID	m_2, M_1, M_2	0-7
Distal Trigonid Crest	DTCREST	m_2, M1, M2	0-1

[a]Maxillary and mandibular arcades are indicated by superscripts and subscripts, respectively. Deciduous and permanent elements are indicated by lowercase letters and uppercase letters, respectively. [b]Grades reflect the Arizona State University Dental Anthropology Syste

Table 2: Deciduous OSF vs. All Other Females

Significance between opposite sex female twins and same sex females, monozygotic females and female siblings comparisons of ASUDAS[1] based on Mann-Whitney-U non-parametric tests, median, sample average, and standard deviation.							
Deciduous	p-value=p(x≤Z)	Median	Median	Sample average	Sample average	Sample SD (S):	Sample SD (S):
	OSF vs SSF+MZ+Sibs	OSF	SSF+MZ+Sibs	OSF	SSF+MZ+Sibs	OSF	SSF+MZ+Sibs
Maxillary							
Canine Shoveling*	0.03	1.50	1.00	1.30	0.82	0.82	0.59
Canine Double- Shoveling	0.07	3.00	2.00	2.78	2.12	1.48	0.90
Canine Tuberculum Dentale*	0.03	1.00	1.00	2.90	1.73	1.91	1.17
Canine Distal Accessory Ridge	0.33	0.00	0.00	0.11	0.24	0.33	0.50
m^2 Metacone (Cusp 3)	0.49	4.00	4.00	3.92	4.00	0.67	0.50
m^2 Hypocone (Cusp 4)	0.370	4.00	4.00	4.17	3.99	0.72	3.99
m^2 Cusp 5 (Metaconule)	0.92	0.00	0.00	0.33	0.29	0.65	0.64
m^2 Carabelli Trait***	0.003	6.00	4.00	5.75	4.41	0.97	1.53
Mandibular							
Canine Distal accessory Ridge	0.19	0.00	0.00	0.14	0.52	0.38	0.63
m_2 Anterior Fovea (Precuspidal Fossa)	0.36	2.00	2.00	2.17	2.32	0.39	0.64
m_2 Cusp Number***	<0.0001	5.00	5.00	5.36	5.09	0.50	0.28
m_2 Deflecting Wrinkle*	0.02	2.00	1.00	1.64	1.05	0.92	0.89
m_2 Protostylid	0.77	4.00	4.00	3.20	3.42	1.93	1.88
m_2 Cusp 5 (Metaconule)	0.47	4.00	4.00	4.08	4.01	0.29	0.44
m_2 Cusp 6 (Tuberculum Sextum)***	<0.0001	0.00	0.00	0.55	0.15	0.82	0.51
m_2 Cusp 7 (Tuberculum Intermedium)	0.53	2.00	1.00	1.42	1.21	0.90	1.06
m_2 Distal Trigonid Crest	0.94	0.00	0.00	0.00	0.09	0.00	0.28
* significant at p < .05; ** significant at p < .0 *** significant at p < 0.003 (Bonferroni correction)							
[1]Arizona State University Dental Anthropology System							

Table 3: Permanent OSF vs. All Other Females

Significance of between opposite sex female twins vs. same sex females, monozygotic females and female siblings. Comparisons of ASUDAS[1] based on Mann-Whitney-U non-parametric tests, median, sample average, standard deviation, and Mood median test.

Permanent Female	p-value=p(x≤Z) OSF vs. MZ+SSF+Sit	Median OSF	Median SSF+MZ+Sibs	Sample average OSF	Sample average SSF+MZ+Sibs	Sample SD (S): OSF	Sample SD (S): SSF+MZ+Sibs
Maxillary							
Canine Shoveling	0.16	1.5	1.0	1.50	1.06	0.58	0.83
Canine Double- Shoveling*	0.05	3.0	2.0	3.00	2.29	0.58	1.11
Canine Tuberculum Dentale	0.80	3.0	3.0	3.25	3.18	0.50	2.27
Canine Distal Accessory Ridge	0.75	3.0	2.0	2.14	1.98	1.46	1.29
M^1 Metacone (Cusp 3)	0.08	5.0	5.0	5.17	4.98	0.39	0.47
M^2 Metacone (Cusp 3)	0.83	4.0	4.5	4.40	4.50	0.55	0.58
M^1 Hypocone (Cusp 4)***	0.002	5.5	5.0	5.50	5.02	0.52	0.65
M^1 Cusp 5 (Metaconule)	0.44	0.0	0.0	0.55	0.34	0.93	0.73
M^2 Cusp 5 (Metaconule)	0.59	0.0	0.0	0.00	0.59	0.00	1.05
M^1 Carabelli Trait*	0.028	6.0	5.0	5.67	4.17	1.23	2.11
M^2 Carabelli Trait	0.13	3.0	1.0	3.20	1.44	2.28	1.89
Mandibular							
Canine Distal accessory Ridge	0.51	0.0	0.0	0.11	0.22	0.33	0.45
Anterior Fovea (Precuspidal Fossa)	0.38	3.0	3.0	2.58	2.39	1.08	1.10
M_1 Cusp Number	0.31	5.0	5.0	5.33	5.10	0.49	0.50
M_2 Cusp Number	0.52	4.0	4.0	4.00	4.28	0.00	0.57
M_1 Deflecting Wrinkle	0.12	1.0	1.0	1.50	1.10	0.90	0.69
M_1 Protostylid	0.76	1.0	1.0	1.27	1.49	1.01	1.33
M_2 Protostylid	0.69	1.0	0.0	0.80	1.08	0.84	1.68
M_1 Cusp 5 (Metaconid)*	0.01	4.0	4.0	3.92	3.15	0.79	1.25
M_2 Cusp 5 (Metaconid)	0.57	0.0	0.0	0.00	0.50	0.00	1.06
M_1 Cusp 6 (Tuberculum Sextum)	0.57	0.0	0.0	0.33	0.27	0.49	0.69
M_1 Cusp 7 (Tuberculum Intermedium)***	<0.0001	0.0	0.0	0.83	0.33	1.53	1.07
M_2 Cusp 7 (Tuberculum Intermedium)	0.61	0.0	0.0	0.43	0.34	0.79	0.92

Mann-Whitney U Monte Carlo * significant at $p < .05$.

*** significant at $p < 0.002$ (Bonferroni correction)

[1]Arizona State University Dental Anthropology System

p-value=p(x≤Z) use Monte Carlo with 10,000 simulations

Table 4: Deciduous All Females vs. Males

Deciduous all female twins and all males including os, ss, mz, and sibs comparisons of ASUDAS[1] based on Mann-Whitney-U non-parametric tests, median, sample average, and standard deviation and Mood median test.

All Females vs All Males Deciduous	Mann-Whitney U	Median Females	Median Males	Mean Female	Mean Male	Standard deviation (n)	Standard deviation (n)
	p-value (Two-tailed)*	n=155	n=129	n=155	n=129	Female	Male
Maxillary							
Canine Shovel	0.003	1.00	1.00	0.86	1.10	0.60	0.62
Canine Double Shovel	0.06	2.00	2.00	2.17	1.91	0.93	0.95
Canine Tuberculum Dentale	0.32	2.00	2.00	1.81	1.95	1.34	1.26
Canine Distal Accessory Ridge	0.80	0.00	0.00	0.23	0.23	0.42	0.49
m^2 Metacone	0.31	4.00	4.00	3.99	4.07	0.72	0.51
m^2 Hypocone	0.38	4.00	4.00	4.00	3.94	0.69	0.63
m^2 C5	0.02	0.00	0.00	0.29	0.24	0.84	0.64
m^2 Carabelli's Trait	0.22	4.00	4.00	4.52	4.28	1.58	1.53
Mandibular							
Canine Distal Accessory Ridge	0.06	0.00	0.00	0.50	0.33	0.59	0.62
m_2 Anterior Fovea	0.12	2.00	2.00	2.31	2.21	0.48	0.63
m_2 Cusp Number	0.18	5.00	5.00	5.11	5.07	0.31	0.31
m_2 Deflecting Wrinkle	0.43	1.00	1.00	1.10	1.19	0.77	0.90
m_2 Protostylid	0.23	3.00	4.00	3.41	3.17	1.64	1.87
m_2 Cusp 5 (Metaconid)	0.71	4.00	4.00	4.01	4.03	0.44	0.43
m_2 Cusp 6 (Tuberculum sextum)	0.13	0.00	0.00	0.18	0.12	0.53	0.54
m_2 Cusp 7 (tuberculum intermedium)	0.65	2.00	2.00	1.22	1.28	1.04	1.05

*Bonferroni Correction 0.003

[1]Arizona State University Dental Anthropology System

p-value = $p(x \leq Z)$ and Mood test use Monte Carlo with 10,000 simulations

Table 5: Permanent All Females vs. All Males

Significance of between all female twins and all males including OS, SS,MZ, and Sibs comparisons of ASUDAS[1] based on Mann-Whitney-U non-parametric tests, median, sample average, and standard deviation and Mood median test.

All Females vs All Males Permanent	Mann-Whitney U	Median Females	Median Males	Mean Female	Mean Male	Standard deviation (n)	Standard deviation (n)
	p-value (Two-tailed)*	n=187	n=144	n=187	n=144	Female	Male
Maxillary							
Canine Shovel	0.68	1.00	1.00	1.14	1.08	0.81	0.80
Canine Double Shovel	0.01	2.00	3.00	2.16	2.59	1.20	1.11
Canine Tuberculum Dentale	0.55	3.00	2.00	3.15	3.02	2.02	2.40
Canine Distal Accessory Ridge*	<0.0001	2.00	3.00	2.06	2.85	1.24	1.22
M^1 Metacone	0.23	5.00	5.00	4.91	4.97	0.51	0.54
M^2 Metacone	0.59	4.00	4.50	4.36	4.37	0.67	0.62
M^1 Hypocone	0.08	5.00	5.00	5.00	5.06	0.60	0.82
M^2 Hypocone	0.64	3.00	3.50	2.81	2.92	1.52	1.62
M^1C5	0.32	0.00	0.00	0.35	0.52	0.77	0.89
M^2C5	0.22	0.00	0.00	0.52	0.32	0.94	0.77
M^1 Carabelli's Trait	0.54	5.00	5.00	4.29	4.47	2.05	1.98
M^2 Carabelli's Trait	0.19	1.00	1.00	1.80	2.23	2.09	2.04
Mandibular							
Canine Distal Accessory Ridge*	<0.0001	0.00	1.00	0.34	1.09	0.66	1.15
M_1 Anterior Fovea	0.50	3.00	3.00	2.39	2.30	1.05	1.07
M_1 Cusp Number	0.65	5.00	5.00	5.17	5.15	0.52	0.44
M_2Cusp Number	0.72	4.00	4.00	4.30	4.29	0.55	0.45
M_1 Deflecting Wrinkle	0.58	1.00	1.00	1.08	1.12	0.75	0.70
M_1Protostylid	0.05	2.00	2.00	1.45	1.82	1.20	1.45
M_2Protostylid	0.73	0.00	0.00	0.85	0.97	1.31	1.45
M_1 Cusp 5 (Metaconid)	0.09	4.00	4.00	3.17	3.42	1.17	1.04
M_2 Cusp 5 (Metaconid)	0.15	0.00	0.00	0.53	0.92	1.04	1.47
M_1 Cusp 6 (Tuberculum sextum)	0.09	0.00	0.00	0.38	0.23	0.80	0.64
M_1 Cusp 7 (tuberculum intermedium)	0.02	0.00	0.00	0.26	0.45	0.93	1.05
M_2 Cusp 7 (tuberculum intermedium)	0.88	0.00	0.00	0.23	0.17	0.71	0.49

*Bonferroni Correction 0.002

[1]Arizona State University Dental Anthropology System

Mann-Whitney U p-value = $p(x \leq Z)$ use Monte Carlo with 10,000 simulations

Table 6: Deciduous OSF vs. OSM

Comparisons of ASUDAS[1] grades osf vs. osm							
Mann-Whitney-U non-parametric tests, median, sample average, and standard deviation.							
Deciduous	p-value=p(x≤Z)	Median	Median	Sample average	Sample average	Sample SD (S)	Sample SD (S)
	osf vs osm	osf	osm	osf	osm	osf	osm
Maxillary							
Canine Shoveling	0.69	1.500	1.000	1.30	1.20	0.82	0.92
Canine Double- Shoveling	0.26	3.00	2.00	2.80	2.27	1.48	0.95
Canine Tuberculum Dentale	0.97	2.50	2.50	2.90	2.88	1.91	1.73
Canine Distal Accessory Ridge	0.11	0.00	0.00	0.11	0.38	0.33	0.52
m^2 Metacone (Cusp 3)	0.97	4.00	4.00	3.92	4.00	0.67	0.95
m^2 Hypocone (Cusp 4)	0.91	4.00	4.00	4.17	4.50	0.72	0.90
m^2 Cusp 5 (Metaconule)	0.91	0.00	0.00	0.27	0.33	0.65	0.47
m^2 Carabelli Trait	0.96	6.00	6.00	5.33	5.75	0.97	2.06
Mandibular							
Canine Distal accessory Ridge	0.53	0.00	0.00	0.14	0.50	0.38	1.22
m_2 Anterior Fovea (Precuspidal Fossa	0.26	2.00	2.00	2.17	1.92	0.39	0.29
m_2 Cusp Number	0.27	5.00	5.00	5.36	5.08	0.50	0.29
m_2 Deflecting Wrinkle	0.09	2.00	1.00	1.64	1.00	0.92	0.85
m_2 Protostylid	0.87	4.00	3.00	3.20	3.45	1.93	1.81
m_2 Cusp 5 (Metaconule)	0.61	4.00	4.00	4.08	4.25	0.29	0.45
m_2 Cusp 6 (Tuberculum Sextum)	0.16	0.00	0.00	0.55	0.08	0.82	0.29
m_2 Cusp 7 (Tuberculum Intermedium)	0.84	2.00	1.50	1.42	1.33	0.90	1.15

Mann-Whitney U with Monte Carlo use 10,000 simulations

[1]Arizona State University Dental Anthropology System

p-value=p(x≤Z)

Table 7: Permanent OSF vs. OSM

Comparisons of ASUDAS[1] grades OSF vs. OSM

Mann-Whitney-U non-parametric tests, median, sample average, and standard deviation.

	Monte Carlo p-value=p(x≤Z) Permanent teeth	Median OSF	Median OSM	Sample average OSF	Sample average OSM	Sample SD (S) OSF	Sample SD (S) OSM
Maxillary							
Canine Shoveling	0.69	1	1	0.85	1.00	0.87	0.71
Canine Double- Shoveling	0.87	3	3	2.54	2.71	0.93	1.14
Canine Tuberculum Dentale	0.60	5	4	4.62	3.83	1.86	2.63
Canine Distal Accessory Ridge**	0.005	0	4	0.58	3.43	1.06	1.30
M^1 Metacone (Cusp 3)	1.00	5	5	5.14	5.07	0.39	0.51
M^2 Metacone (Cusp 3)	0.74	5	5	4.64	4.86	0.53	0.45
M^1 Hypocone (Cusp 4)	0.49	5	5.5	5.21	5.50	0.62	0.52
M^1 Cusp 5 (Metaconule)	0.67	0	0	0.29	0.23	0.78	0.65
M^1 Carabelli Trait	0.86	5	6	4.86	5.14	2.12	2.37
M^1 Carabelli Trait	0.75	1	1	1.64	1.29	1.86	1.14
Mandibular							
Canine Distal accessory Ridge	0.08	0	1	0.15	1.09	0.40	1.27
Anterior Fovea (Precuspidal Fossa)	0.85	2	3	2.21	2.38	1.29	1.10
M_1 Cusp Number	0.69	5	5	5.00	5.17	0.74	0.63
M_2 Cusp Number	0.55	4	4	4.27	4.14	0.71	0.45
M_1 Deflecting Wrinkle	0.72	1	1	1.15	0.93	1.17	0.90
M_1 Protostylid	0.11	1	2	1.00	1.92	0.94	1.14
M_2 Protostylid	0.74	0	1.5	0.44	1.33	0.79	0.96
M_1 Cusp 5 (Hypoconulid)	0.39	4	4	2.86	3.42	1.83	1.49
M_2 Cusp 5 (Hypoconulid)	0.55	0	0	0.55	0.14	1.32	0.45
M_1 Cusp 6 (Tuberculum Sextum)	0.63	0	0	0.43	0.73	1.17	1.36
M_2 Cusp 7 (Tuberculum Intermedium)	0.31	0	0	0.33	0.00	0.84	0.00

** significant at $p < .01$.

*** none were significant at $p < 0.002$ (Bonferroni correction)

[1]Arizona State University Dental Anthropology System

Figure (1): The tooth germ stages of deciduous canines and molars along with permanent canines and first and second molar (dates from Kitamura, 1989) and the testosterone levels (from Tapp *et al.*, 2011) set against fetal age (Reyes *et al.*, 1974; Knickmeyer and Baron-Cohen, 2006).

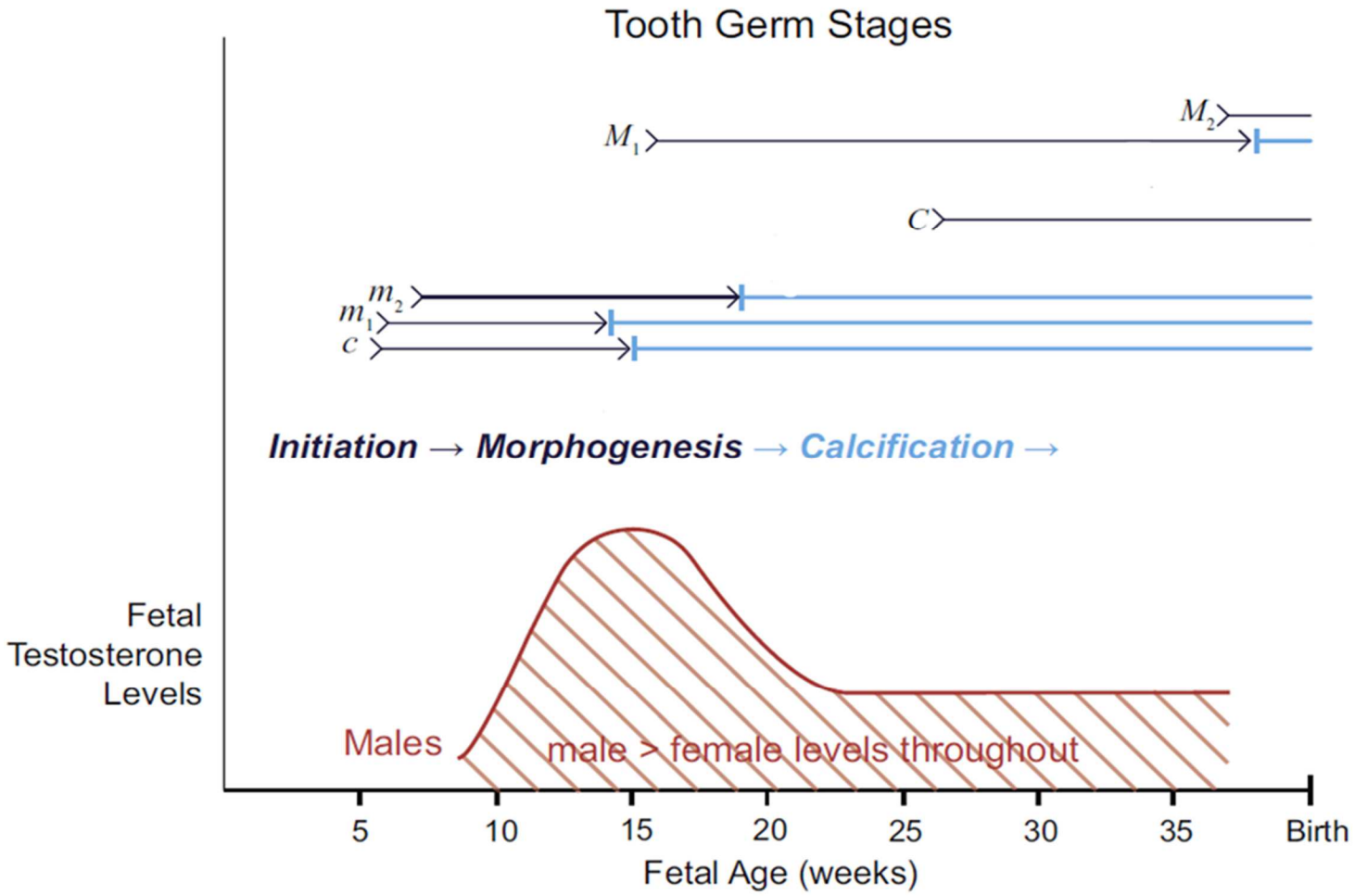
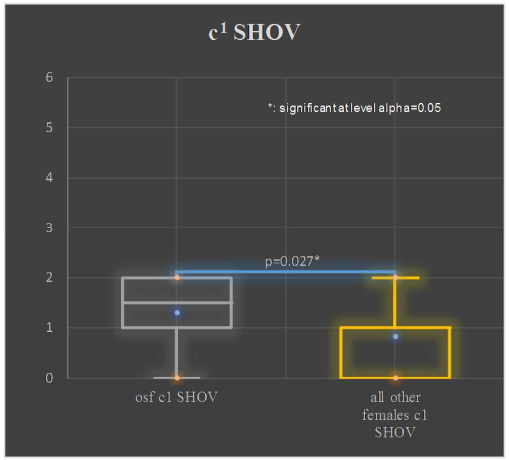
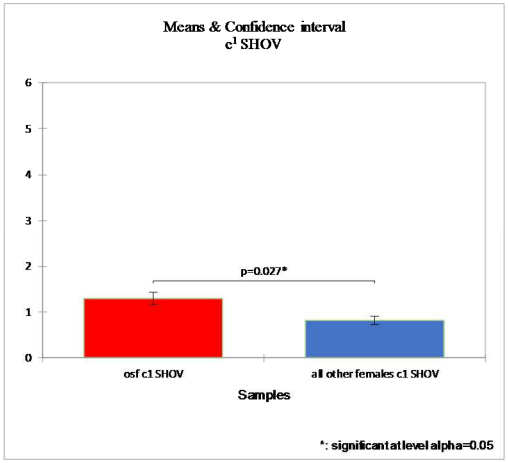


Figure 2: Deciduous c^1 SHOV



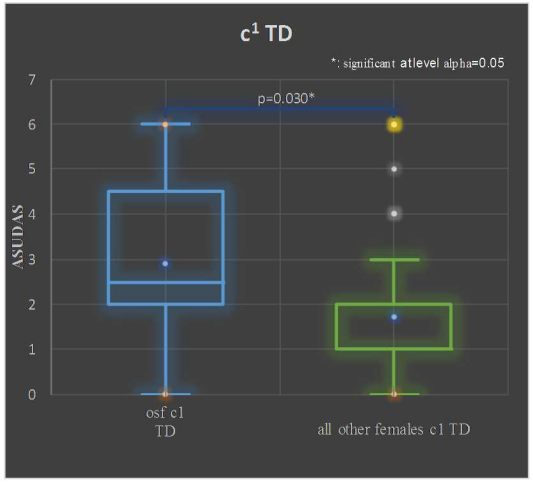


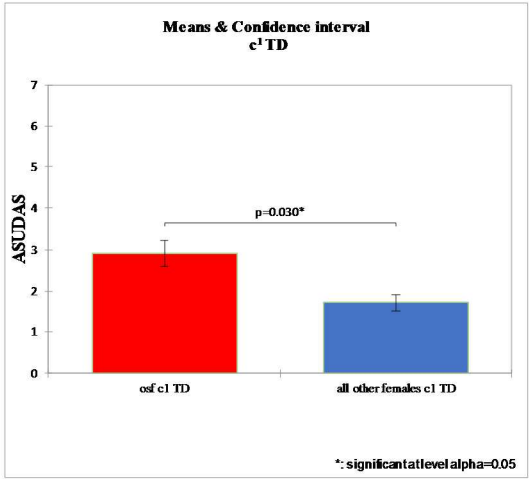
Mann-Whitney test / Two-tailed test:

U	830
Expected value	610.000
Variance (U)	10320.472
p-value (Two-tailed)	**0.027**
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 3: Deciduous c^1 TD



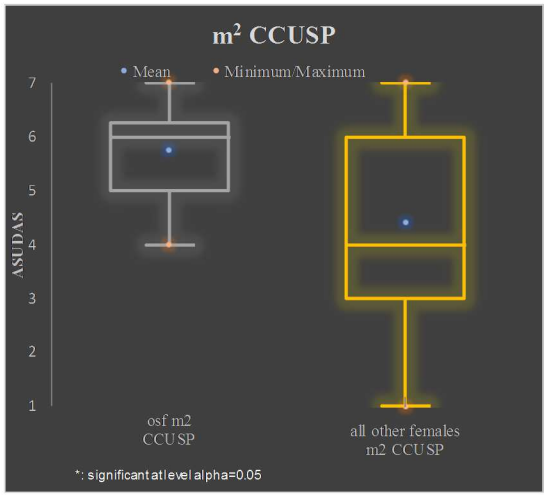


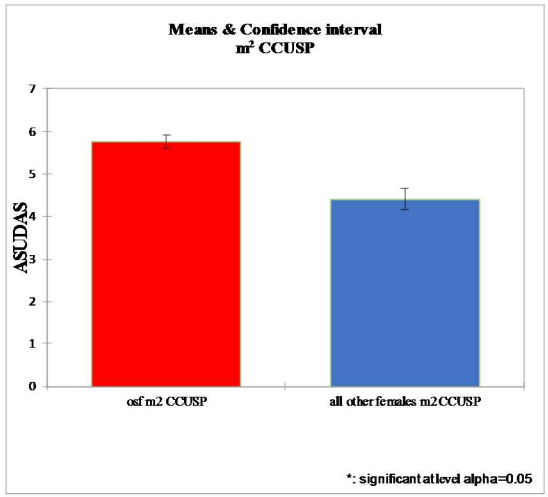
Mann-Whitney test / Two-tailed test:

U	856
Expected value	615.000
Variance (U)	12450.177
p-value (Two-tailed)	**0.030**
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 4: Deciduous m^2 CCUSP



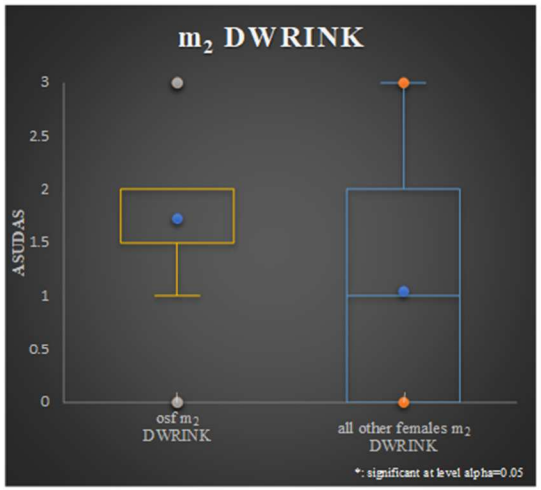


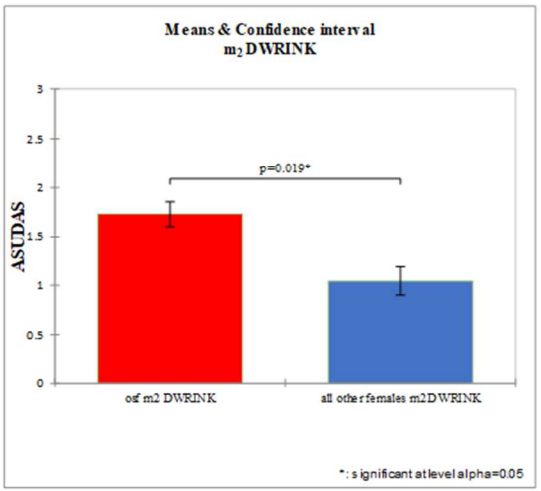
Mann-Whitney test / Two-tailed test:

U	1244
Expected value	822.000
Variance (U)	19826.631
p-value (Two-tailed)	**0.003**
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 5: Deciduous m_2 DWRINK



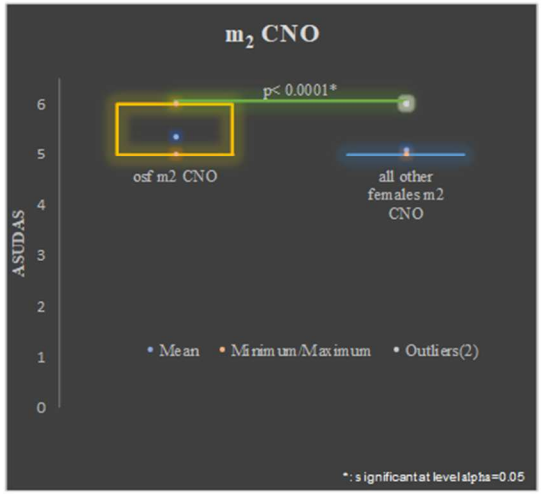


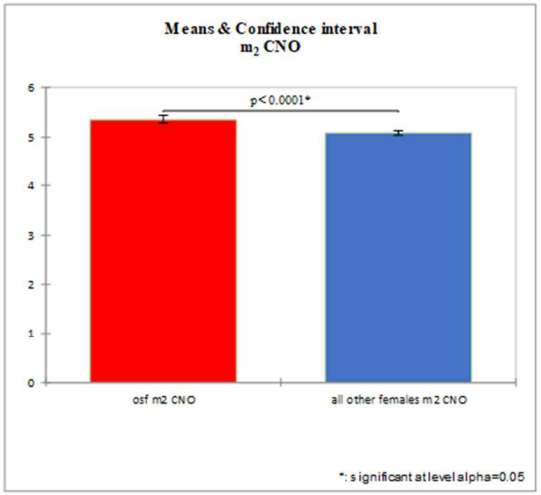
Mann-Whitney test / Two-tailed test:

U	1015.500
Expected value	720.500
Variance (U)	15327.700
p-value (Two-tailed)	**0.019**
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 6: Deciduous m_2 CNO



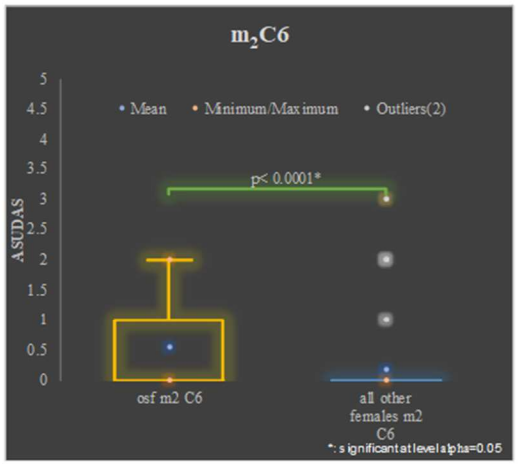


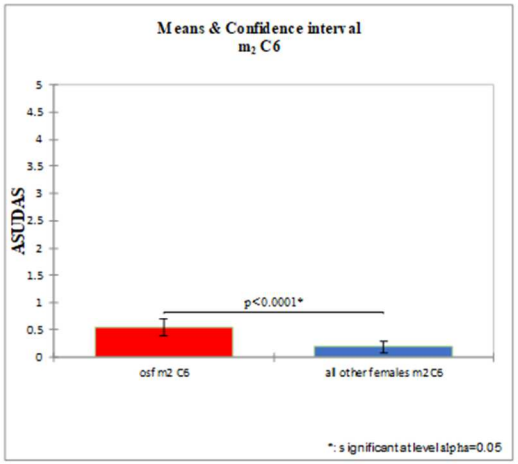
Mann-Whitney test / Two-tailed test:

U	969
Expected value	759.000
Variance (U)	5456.595
p-value (Two-tailed)	**<0.0001**
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 7: Deciduous m_2 C6



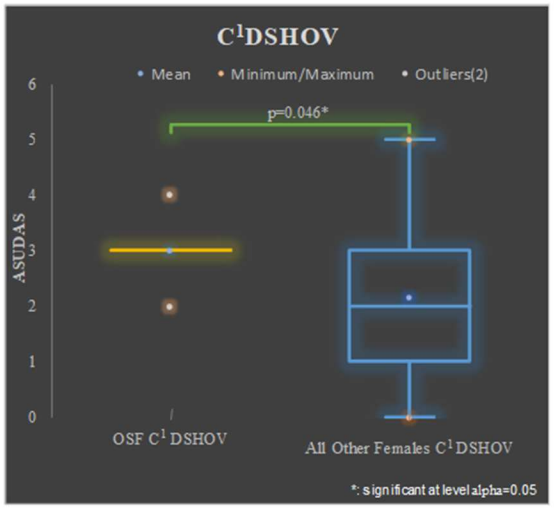


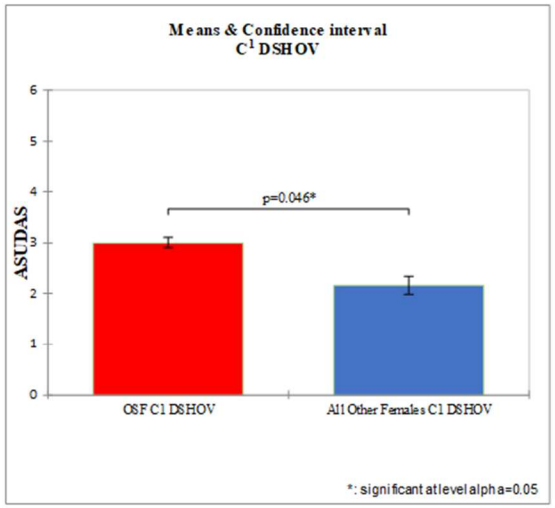
Mann-Whitney test / Two-tailed test:

U	715.500
Expected value	572.000
Variance (U)	3782.399
p-value (Two-tailed)	**<0.0001**
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 8: Permanent C^1 DSHOV



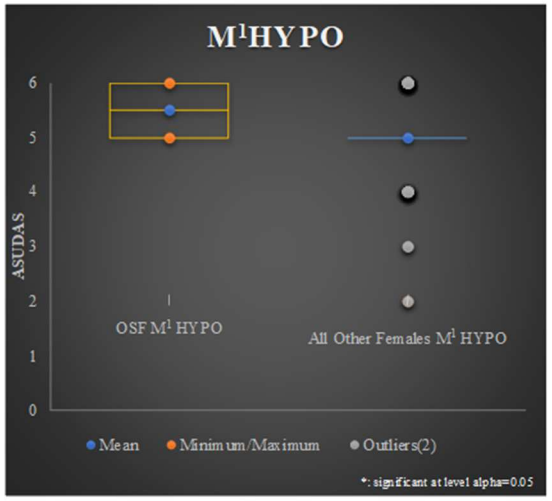


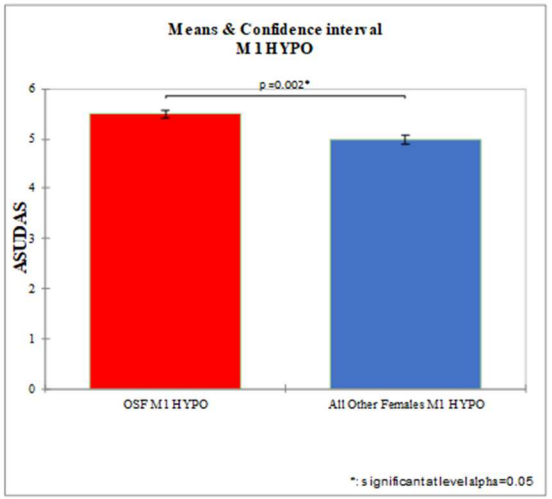
Mann-Whitney test / Two-tailed test:

U	511.500
Expected value	348.000
Variance (U)	6642.222
p-value (Two-tailed)	**0.046**
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 9: Permanent M^1 HYPO



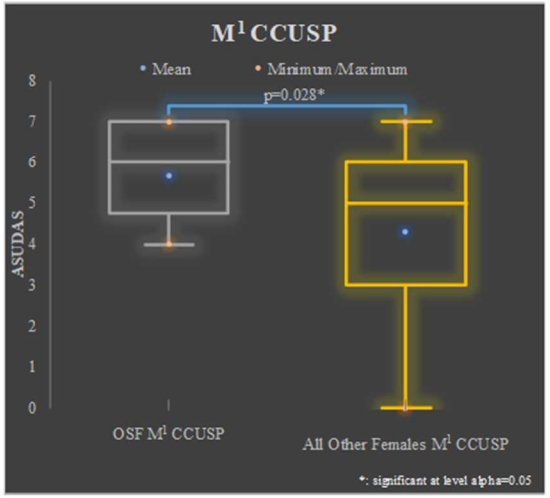


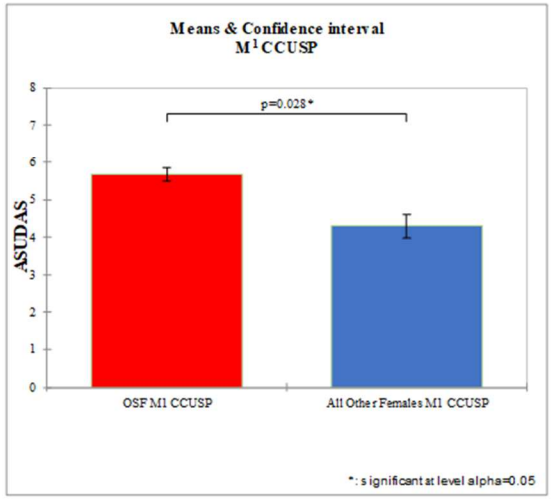
Mann-Whitney test / Two-tailed test:

U	1368
Expected value	966.000
Variance (U)	17293.043
p-value (Two-tailed)	**0.002**
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 10: Permanent M^1 CCUSP



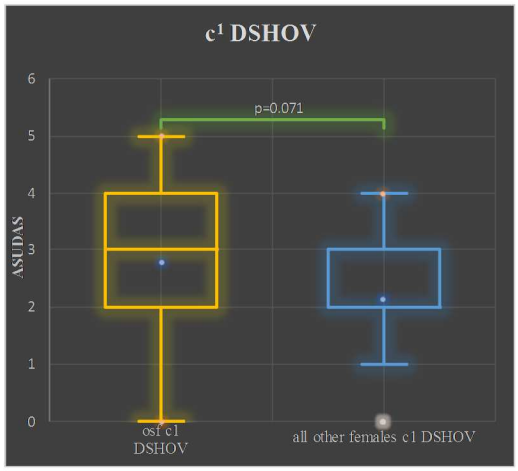


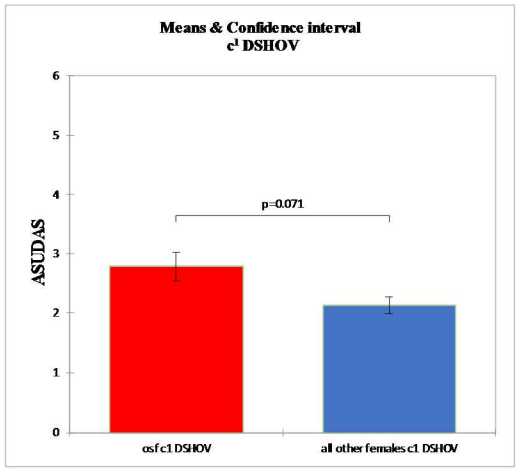
Mann-Whitney test / Two-tailed test:

U	1348
Expected value	978.000
Variance (U)	27419.330
p-value (Two-tailed)	**0.028**
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 11: Deciduous c^1 DSHOV



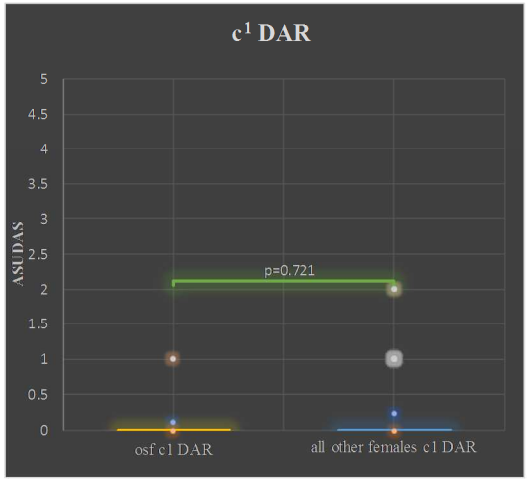


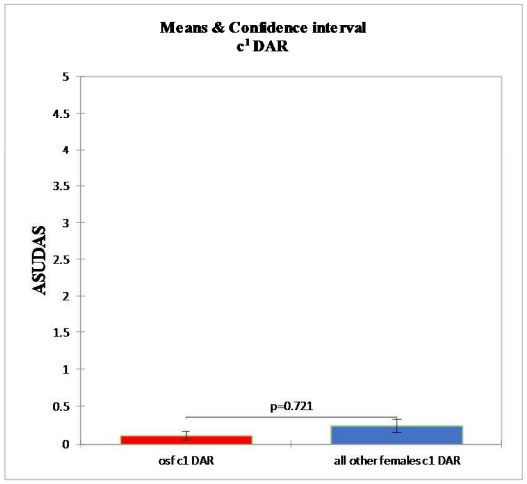
Mann-Whitney test / Two-tailed test:

U	759
Expected value	576.000
Variance (U)	11231.536
p-value (Two-tailed)	0.071
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 12: Deciduous c^1 DAR



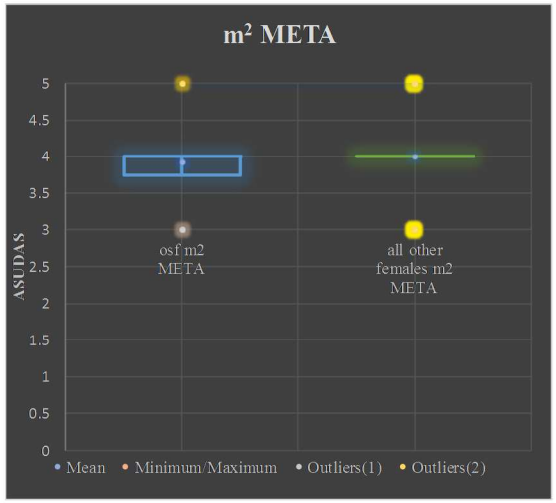


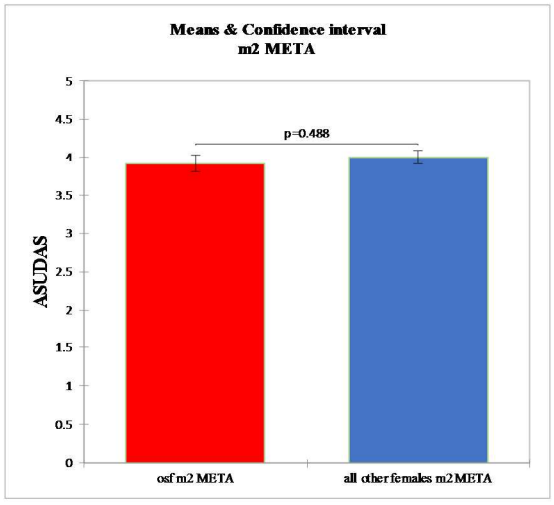
Mann-Whitney test / Two-tailed test:

U	378.500
Expected value	423.000
Variance (U)	3593.447
p-value (Two-tailed)	0.721
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 13: Deciduous m^2 META



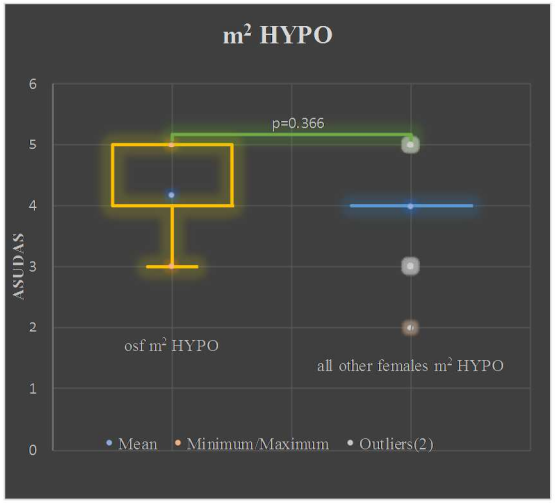


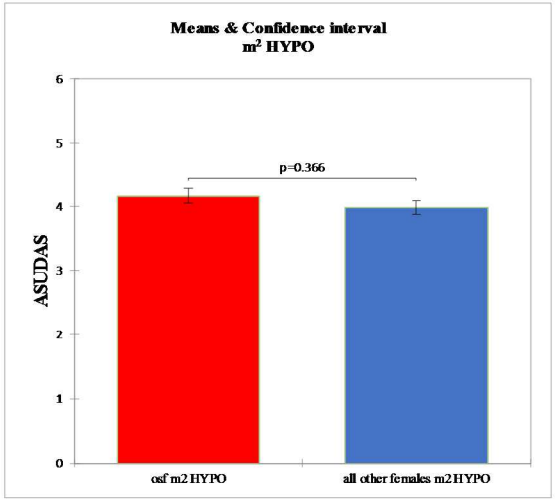
Mann-Whitney test / Two-tailed test:

U	756.500
Expected value	816.000
Variance (U)	12076.620
p-value (Two-tailed)	0.488
alpha	0.050

The p-value has been computed . using 10000 Monte Carlo simulations.

Figure 14: Deciduous m2 HYPO



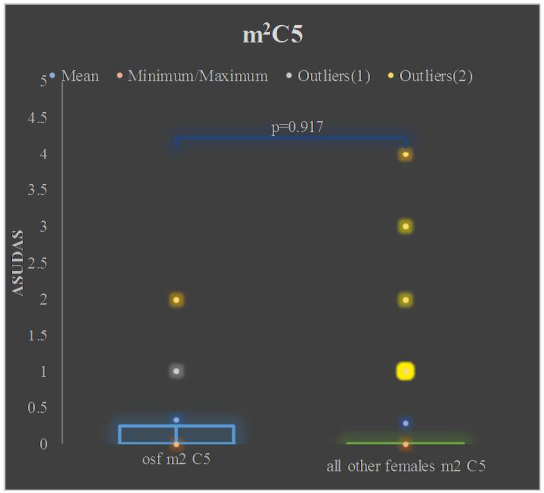


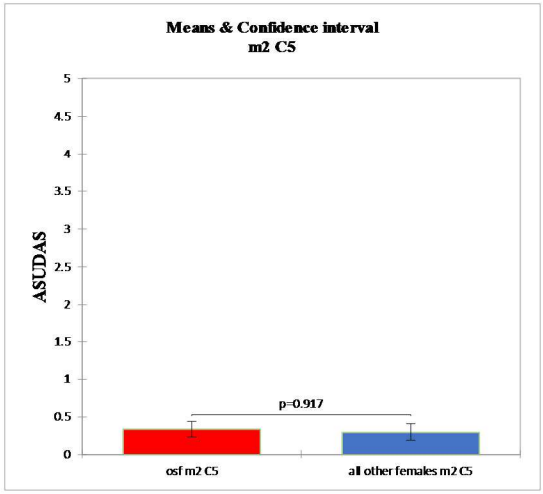
Mann-Whitney test / Two-tailed test:

U	928
Expected value	816.000
Variance (U)	14378.568
p-value (Two-tailed)	0.366
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 15: Deciduous m^2 C5



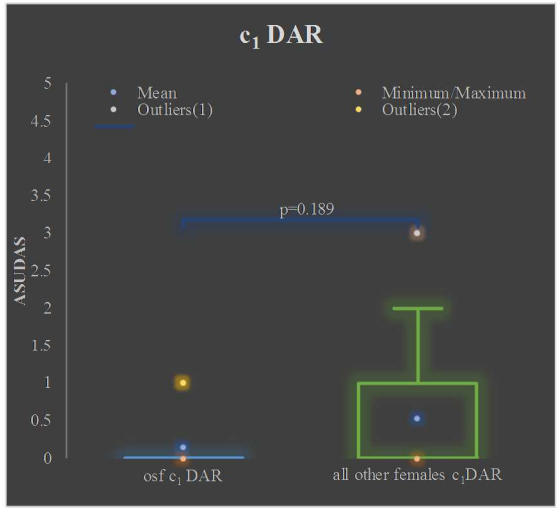


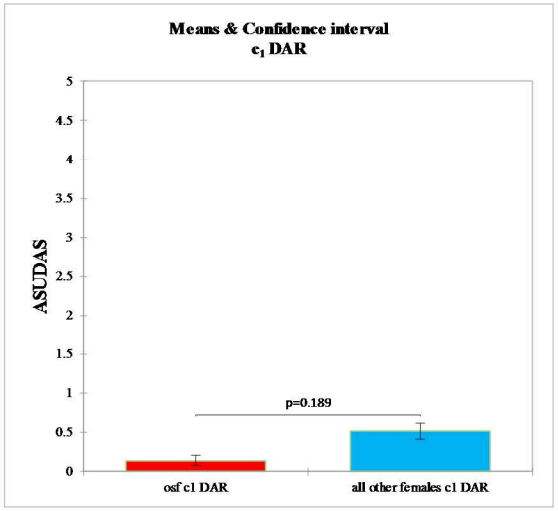
Mann-Whitney test / Two-tailed test:

U	831
Expected value	804.000
Variance (U)	10440.986
p-value (Two-tailed)	0.917
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 16: Deciduous c_1 DAR



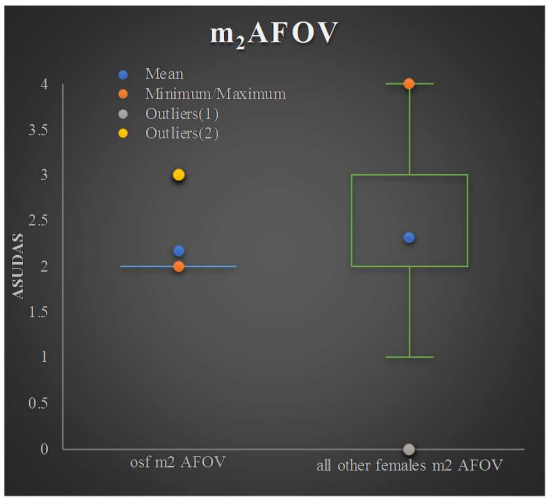


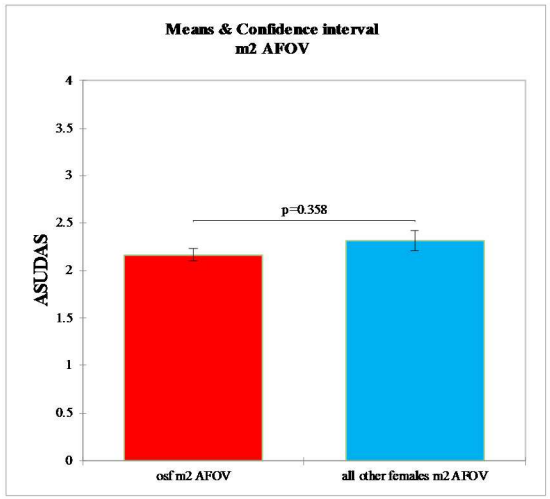
Mann-Whitney test / Two-tailed test:

U	223
Expected value	329.000
Variance (U)	4265.729
p-value (Two-tailed)	0.189
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 17: Deciduous m_2 AFOV



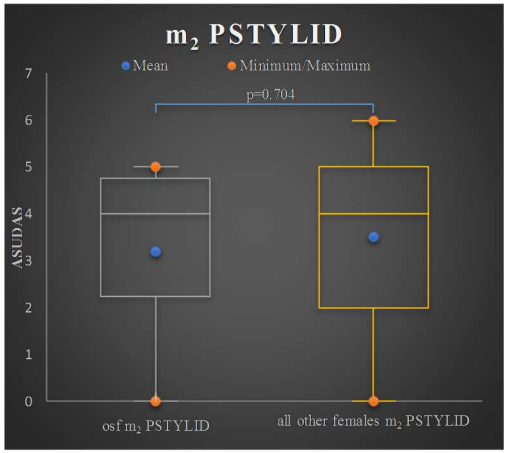


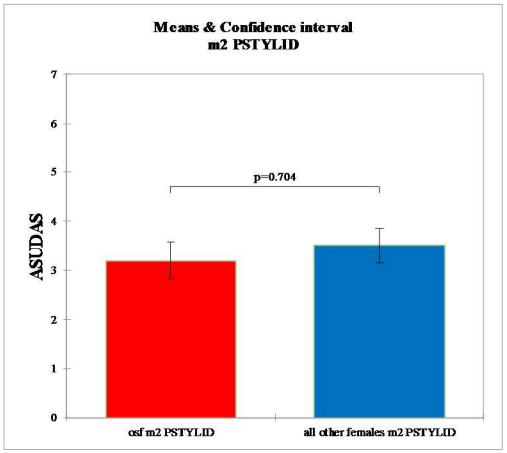
Mann-Whitney test / Two-tailed test:

U	680
Expected value	798.000
Variance (U)	14560.748
p-value (Two-tailed)	0.358
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 18: Deciduous m_2 PSTYLID



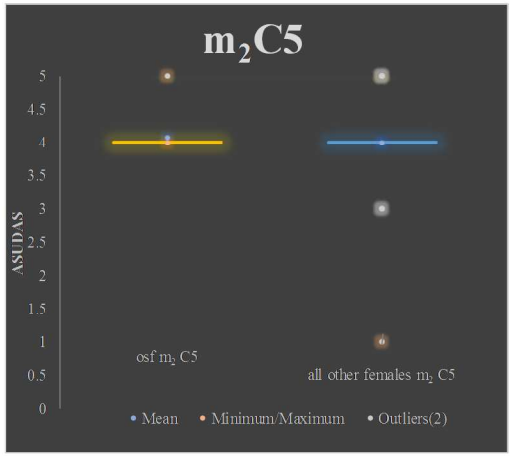


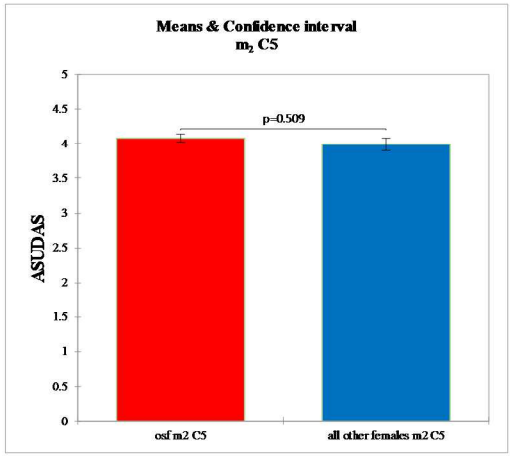
Mann-Whitney test / Two-tailed test:

U	475.500
Expected value	515.000
Variance (U)	9496.452
p-value (Two-tailed)	0.704
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 19: Deciduous m_2 C5



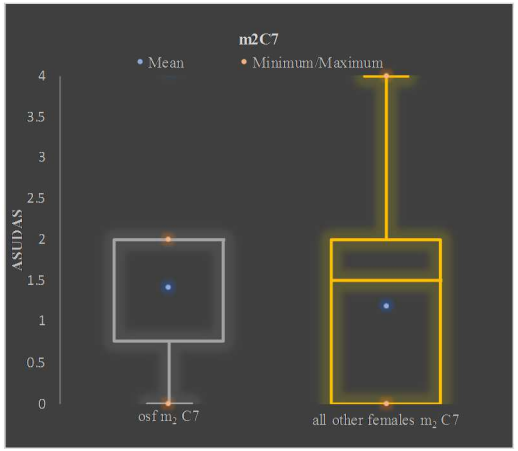


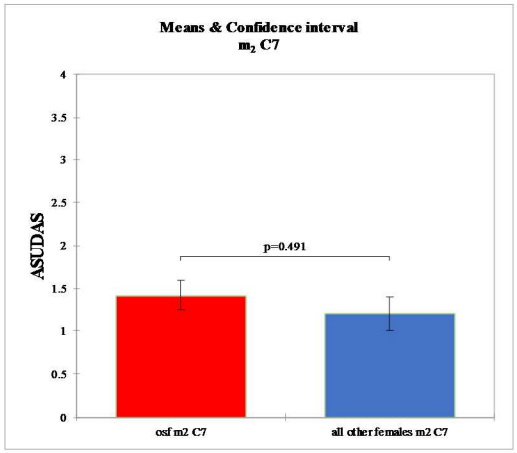
Mann-Whitney test / Two-tailed test:

U	667
Expected value	624.000
Variance (U)	3890.645
p-value (Two-tailed)	0.509
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 20: Deciduous m_2 C7



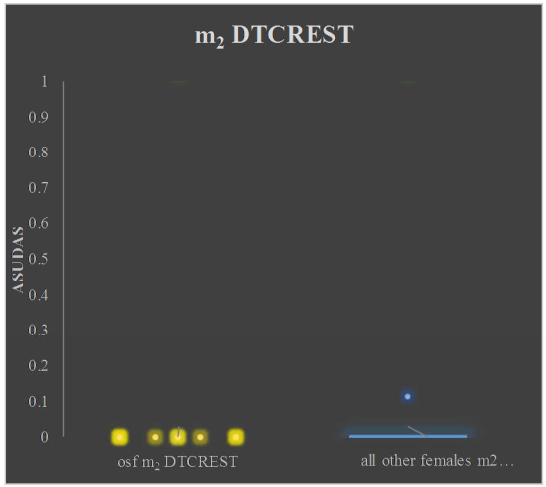


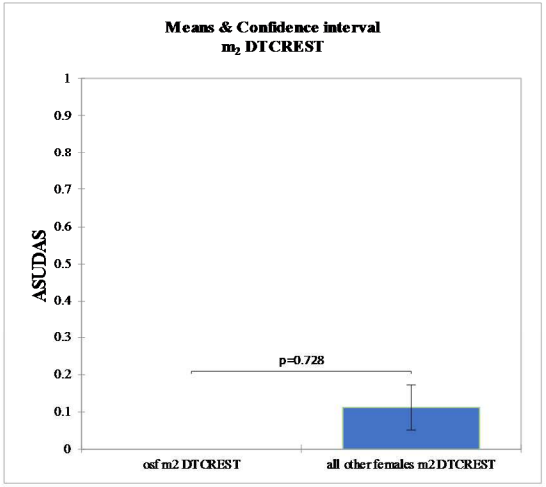
Mann-Whitney test / Two-tailed test:

U	714.500
Expected value	636.000
Variance (U)	10772.751
p-value (Two-tailed)	0.491
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 21: Deciduous m_2 DTCREST



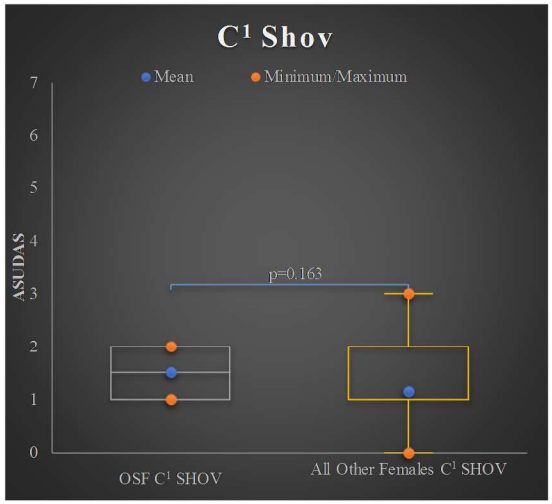


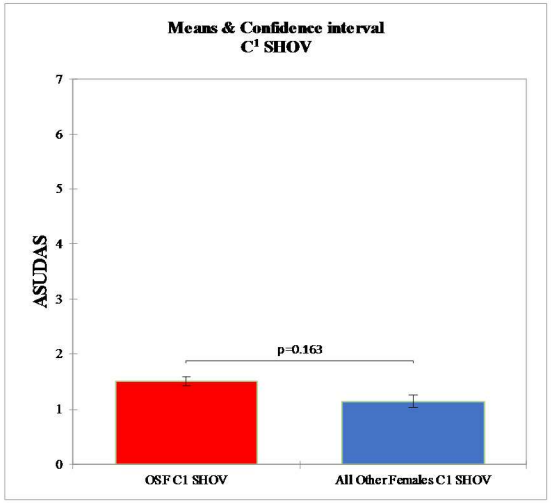
Mann-Whitney test / Two-tailed test:

U	391.500
Expected value	441.000
Variance (U)	2196.679
p-value (Two-tailed)	0.728
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 22: Permanent C[1]SHOV



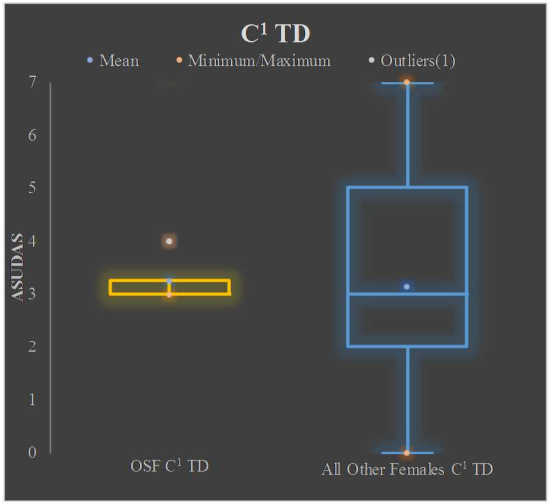


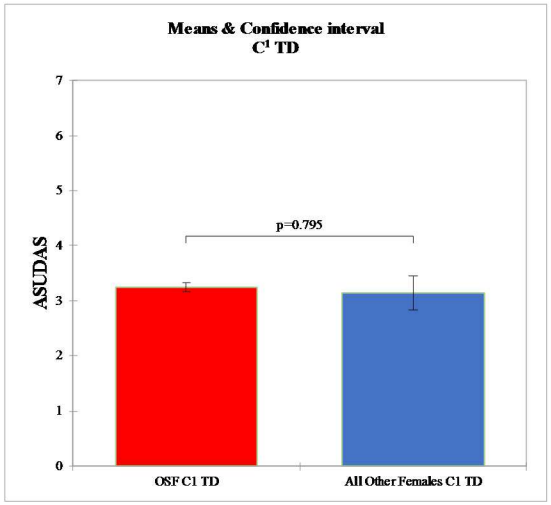
Mann-Whitney test / Two-tailed test:

U	232
Expected value	180.000
Variance (U)	2394.049
p-value (Two-tailed)	0.163
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 23: Permanent C[1] TD



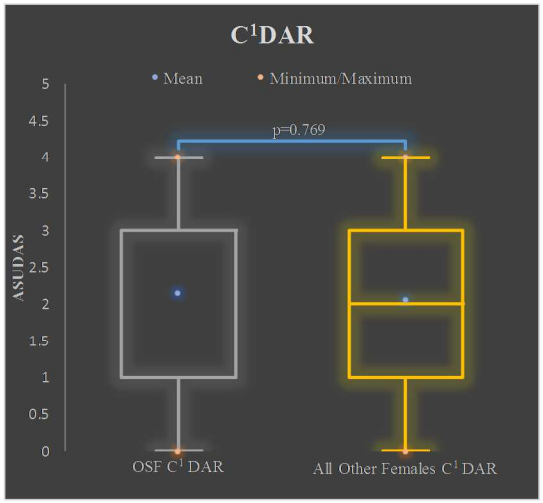


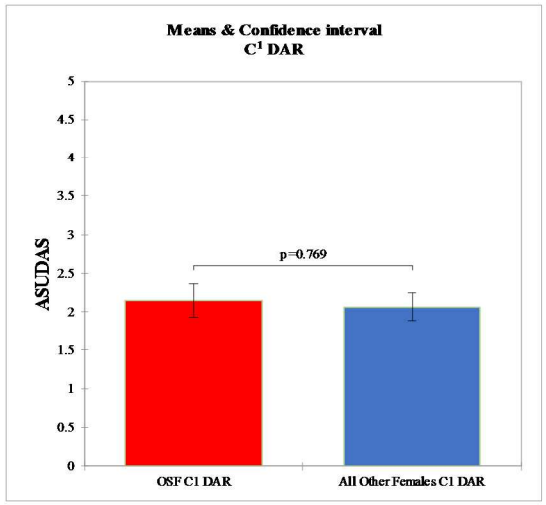
Mann-Whitney test / Two-tailed test:

U	189.500
Expected value	174.000
Variance (U)	2609.596
p-value (Two-tailed)	0.795
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 24: Permanent C^1 DAR



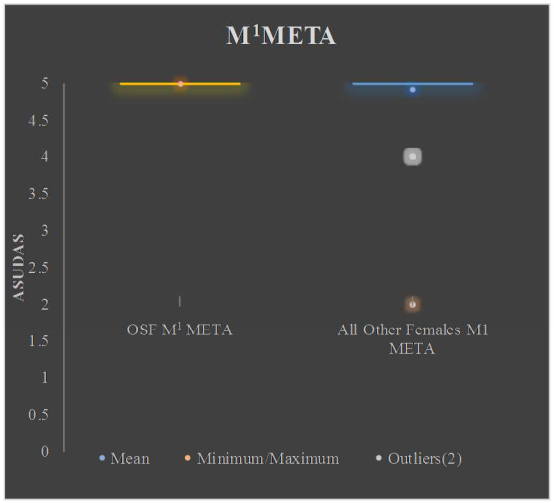


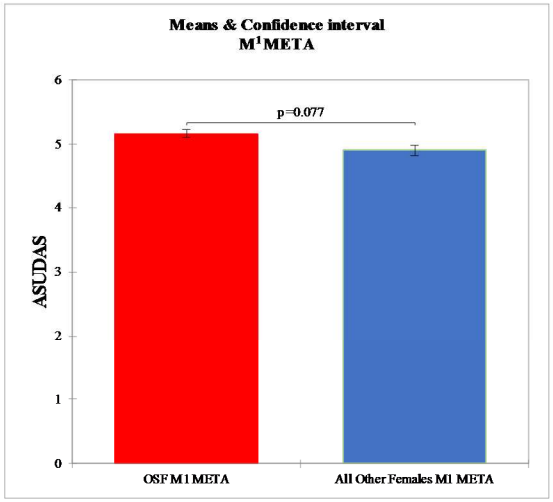
Mann-Whitney test / Two-tailed test:

U	409.500
Expected value	385.000
Variance (U)	6958.764
p-value (Two-tailed)	0.769
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 25: Permanent M^1 META



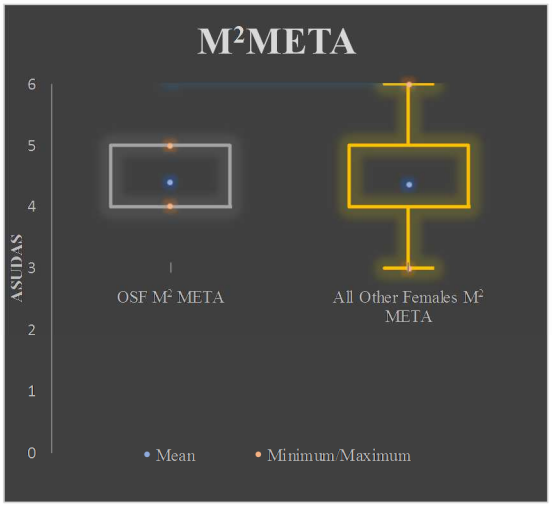


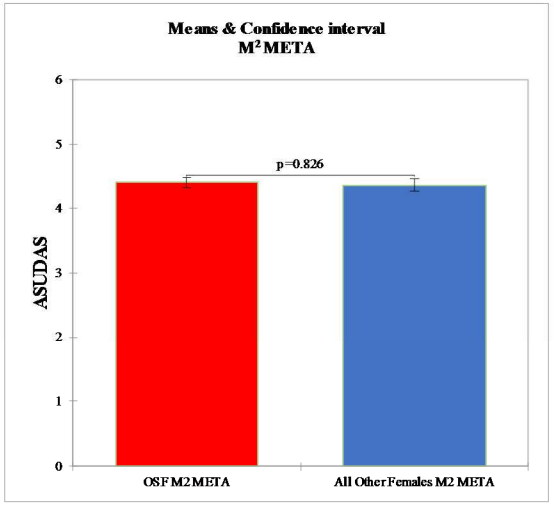
Mann-Whitney test / Two-tailed test:

U	1222
Expected value	1002.000
Variance (U)	15591.821
p-value (Two-tailed)	0.077
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 26: Permanent M^2 META





Mann-Whitney test / Two-tailed test:

U	239
Expected value	237.500
Variance (U)	3289.375
p-value (Two-tailed)	0.826
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 27: Permanent (One observation OSF M^2 Hypocone precludes bar graph.)

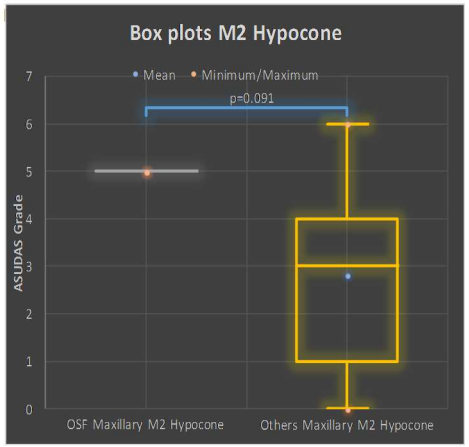
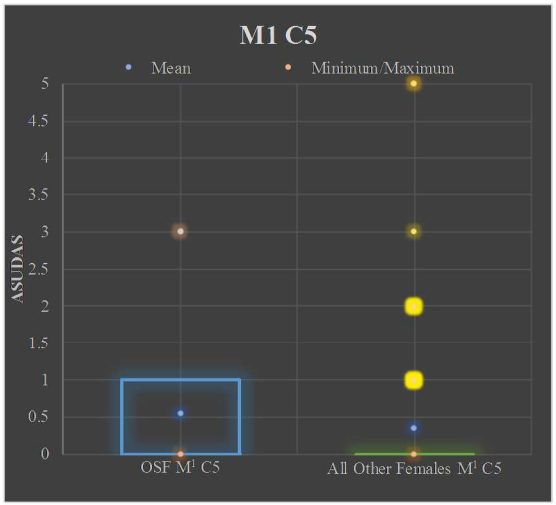
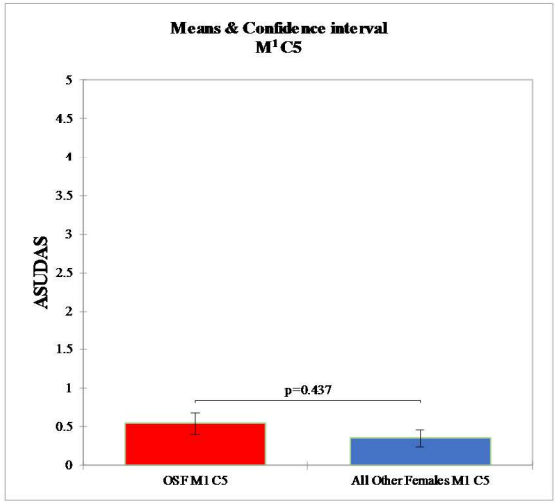


Figure 28: Permanent M^1 C5



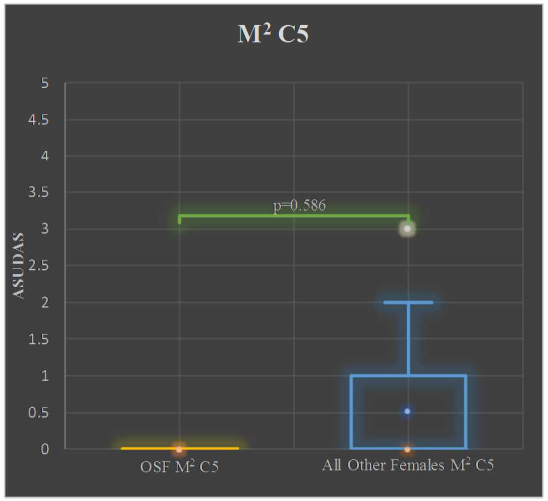


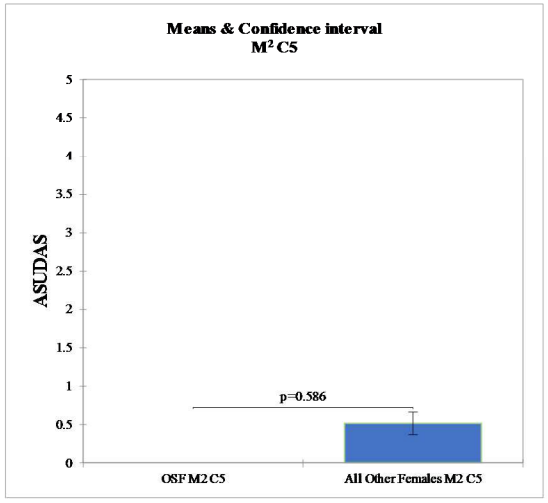
Mann-Whitney test / Two-tailed test:

U	977
Expected value	863.500
Variance (U)	13242.334
p-value (Two-tailed)	0.437
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 29: Permanent M^2 C5



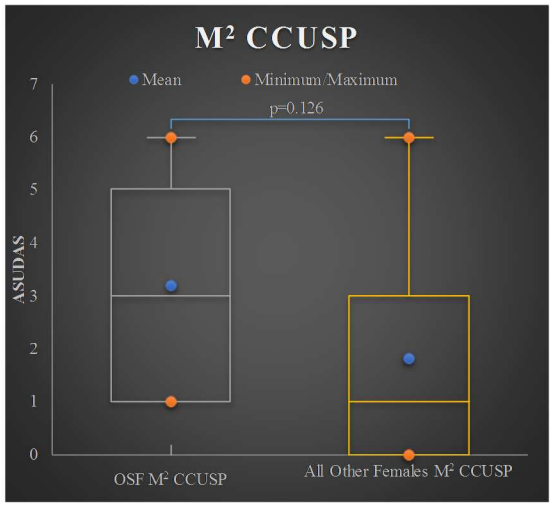


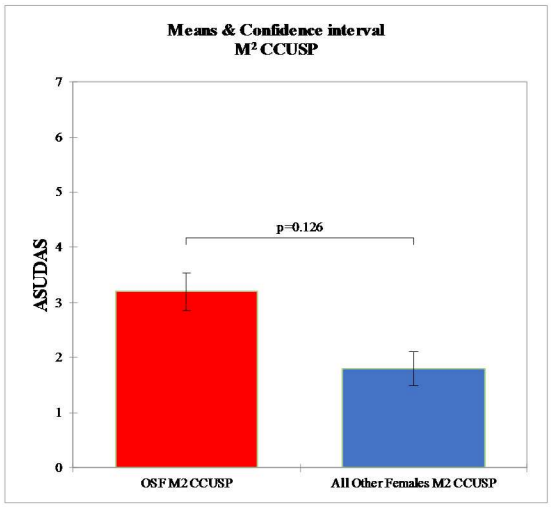
Mann-Whitney test / Two-tailed test:

U	96
Expected value	132.000
Variance (U)	918.261
p-value (Two-tailed)	0.586
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 30: Permanent M^2 CCUSP



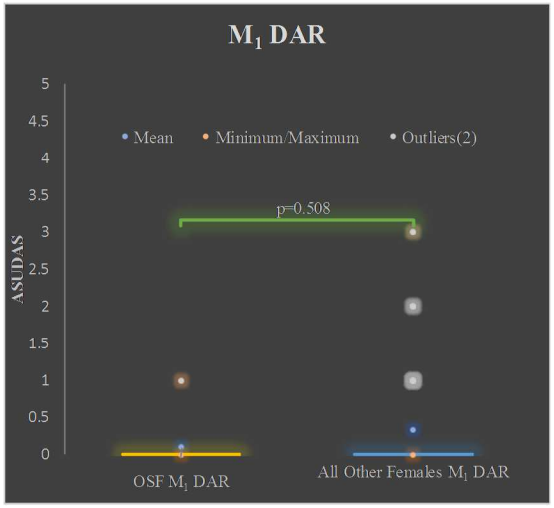


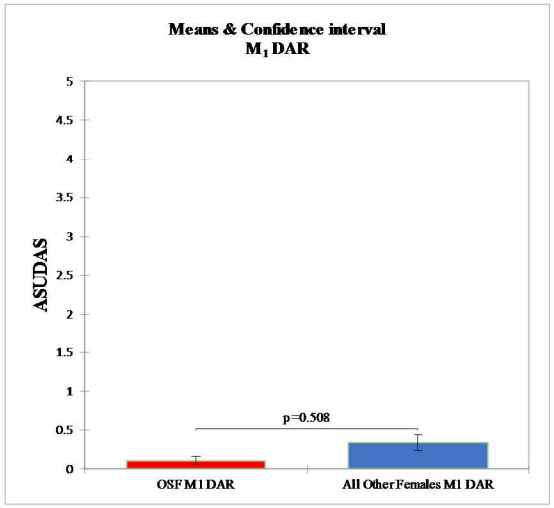
Mann-Whitney test / Two-tailed test:

U	299.500
Expected value	210.000
Variance (U)	2938.847
p-value (Two-tailed)	0.126
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 31: Permanent M_1 DAR



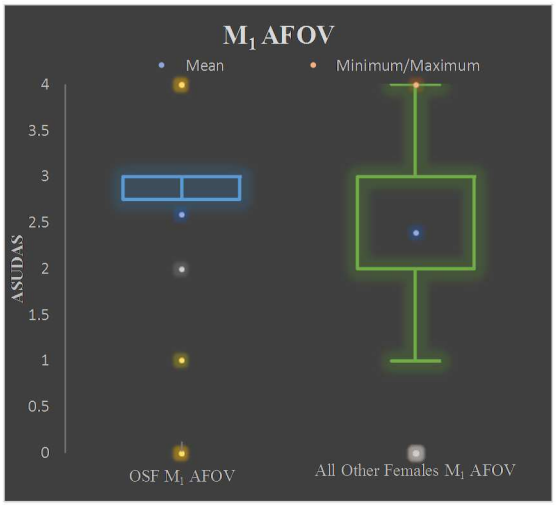


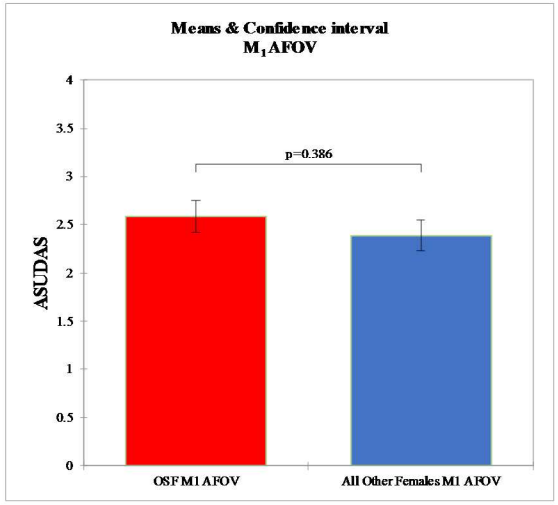
Mann-Whitney test / Two-tailed test:

U	469.500
Expected value	544.500
Variance (U)	6431.399
p-value (Two-tailed)	0.508
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 32: Permanent M_1 AFOV



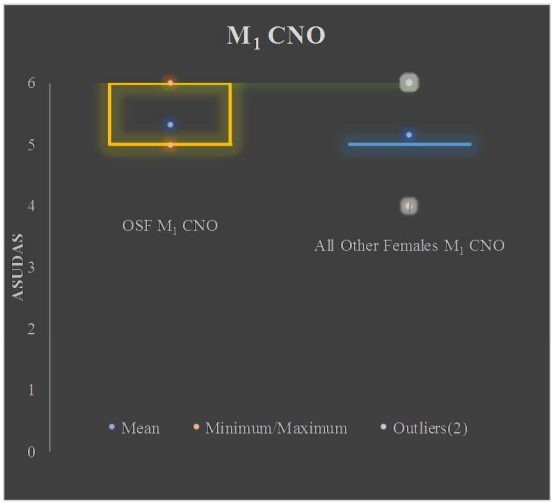


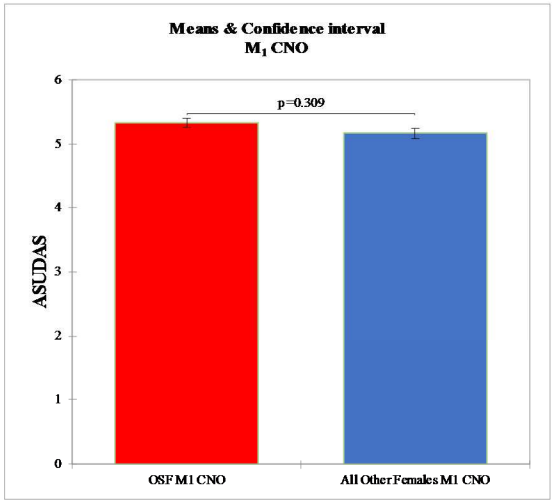
Mann-Whitney test / Two-tailed test:

U	1031
Expected value	900.000
Variance (U)	22187.440
p-value (Two-tailed)	0.386
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 33: Permanent M_1 CNO



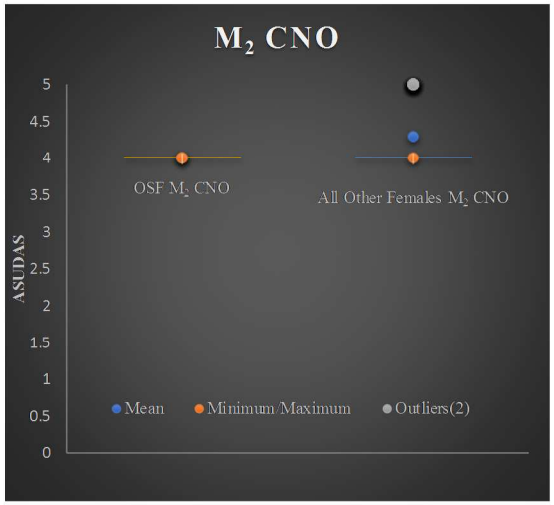


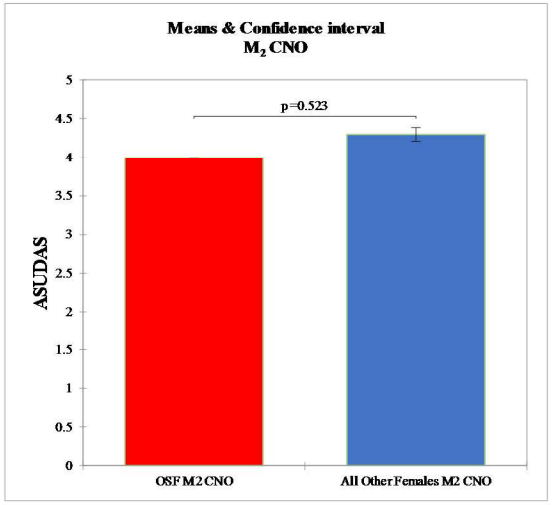
Mann-Whitney test / Two-tailed test:

U	992
Expected value	870.000
Variance (U)	14701.835
p-value (Two-tailed)	0.309
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 34: Permanent M_2 CNO



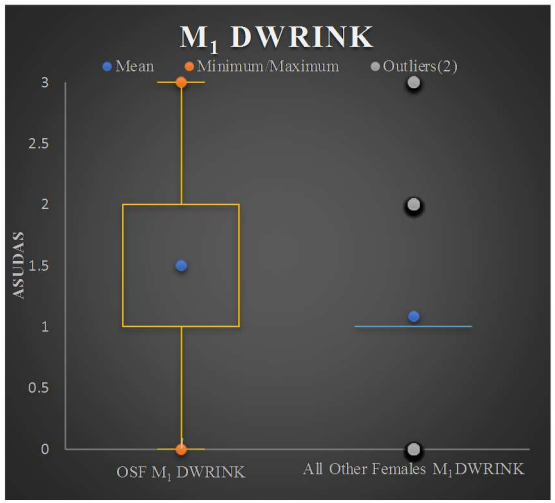


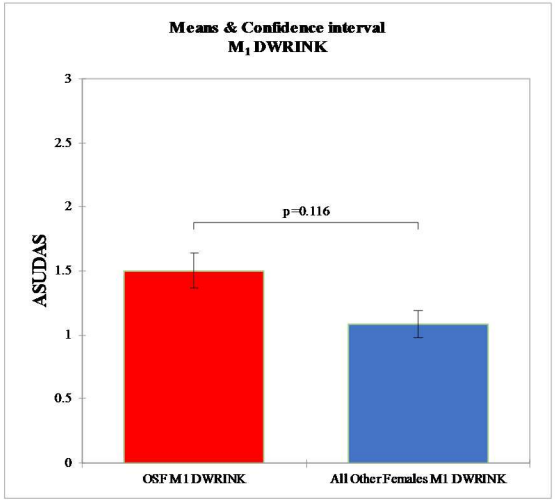
Mann-Whitney test / Two-tailed test:

U	137.500
Expected value	182.500
Variance (U)	1295.185
p-value (Two-tailed)	0.523
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 35: Permanent M_1 DWRINK



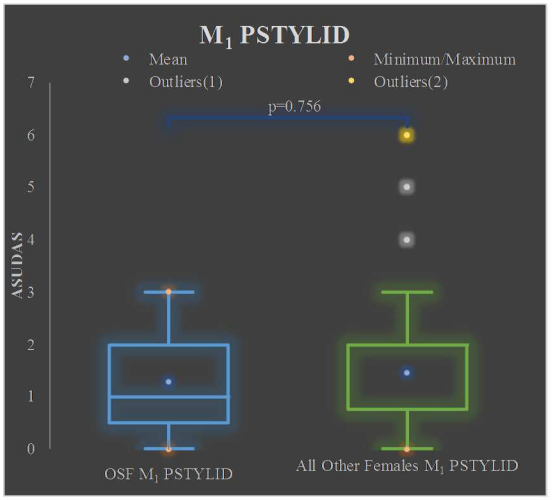


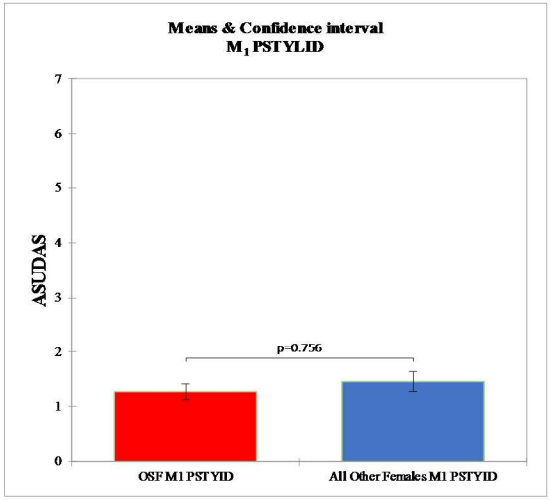
Mann-Whitney test / Two-tailed test:

U	1167.500
Expected value	930.000
Variance (U)	20324.368
p-value (Two-tailed)	0.116
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 36: Permanent M_1 PSTYLID



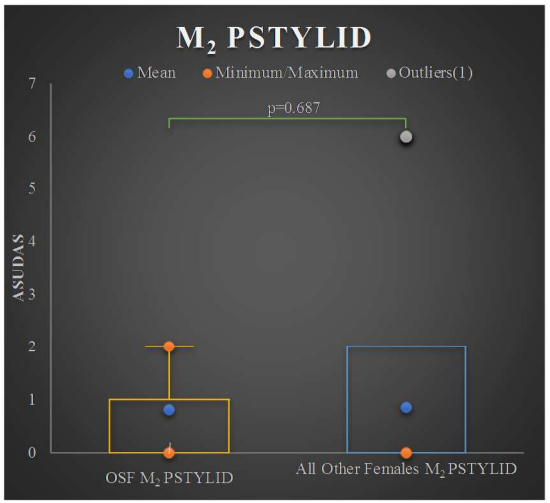


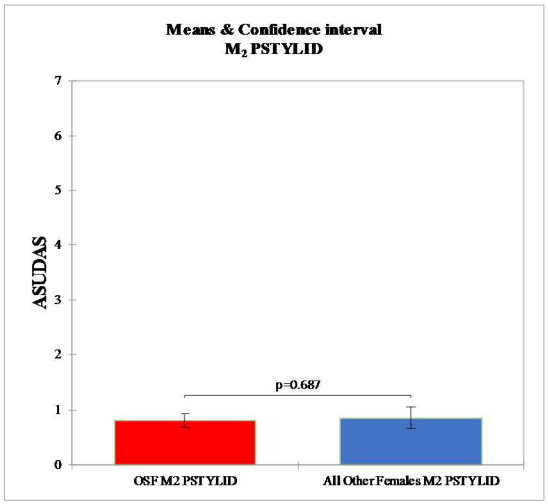
Mann-Whitney test / Two-tailed test:

U	771.500
Expected value	814.000
Variance (U)	19556.316
p-value (Two-tailed)	0.756
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 37: Permanent M_2 PSTYLID



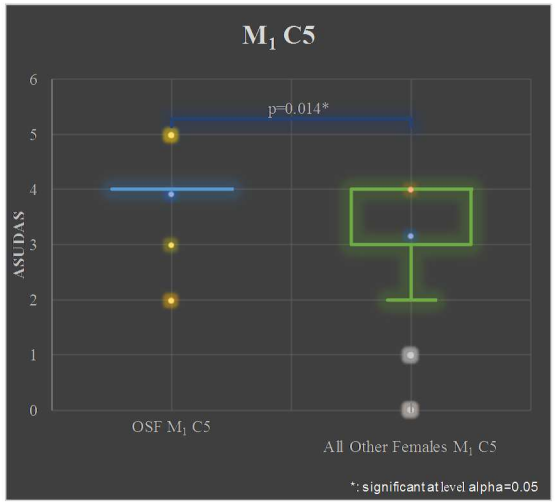


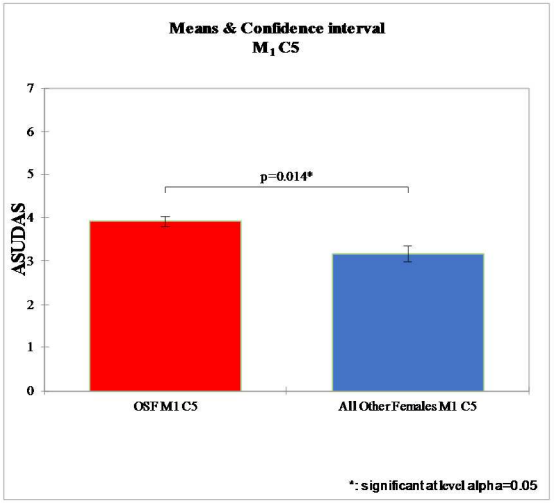
Mann-Whitney test / Two-tailed test:

U	223.500
Expected value	205.000
Variance (U)	2299.387
p-value (Two-tailed)	0.687
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 38: Permanent M_1 C5



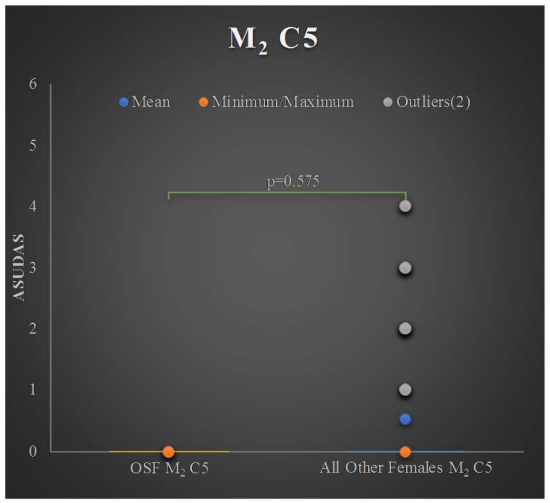


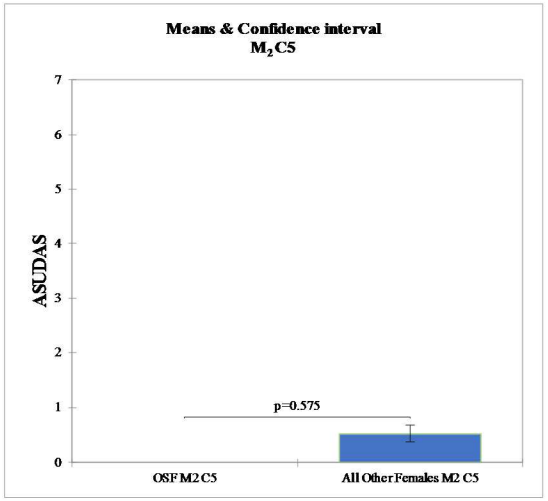
Mann-Whitney test / Two-tailed test:

U	1204.500
Expected value	870.000
Variance (U)	18667.134
p-value (Two-tailed)	**0.014**
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 39: Permanent M_2 C5



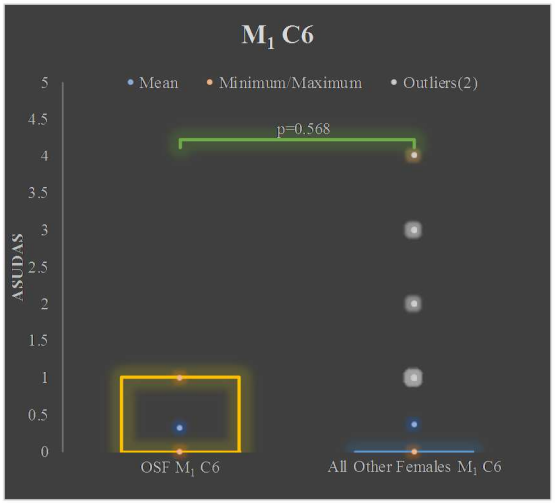


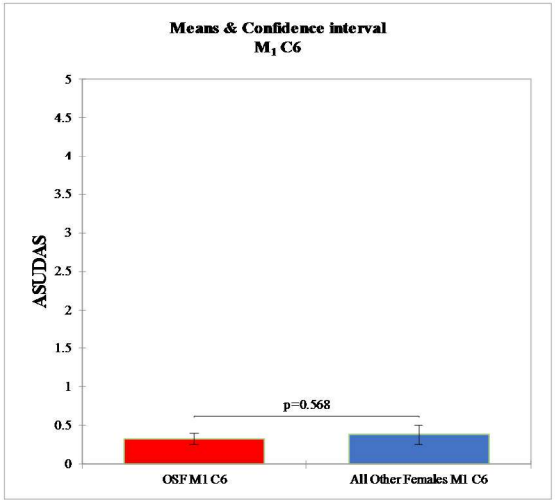
Mann-Whitney test / Two-tailed test:

U	142.500
Expected value	185.000
Variance (U)	1272.453
p-value (Two-tailed)	0.575
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 40: Permanent M_1 C6



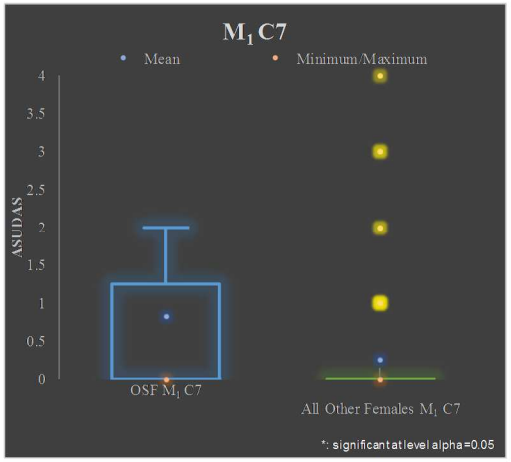


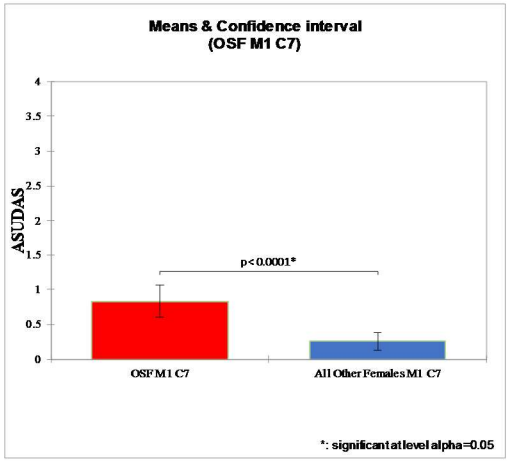
Mann-Whitney test / Two-tailed test:

U	936
Expected value	870.000
Variance (U)	12596.031
p-value (Two-tailed)	0.568
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 41: Permanent M_1 C7



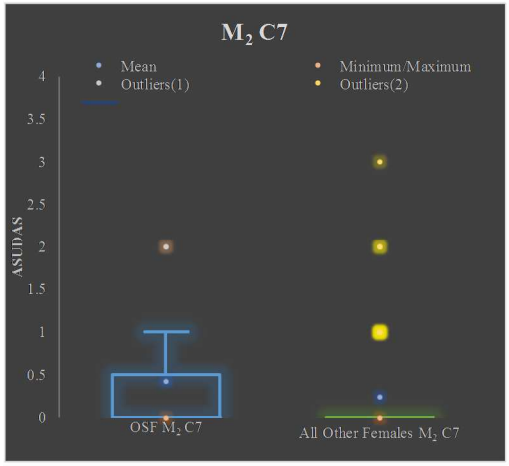


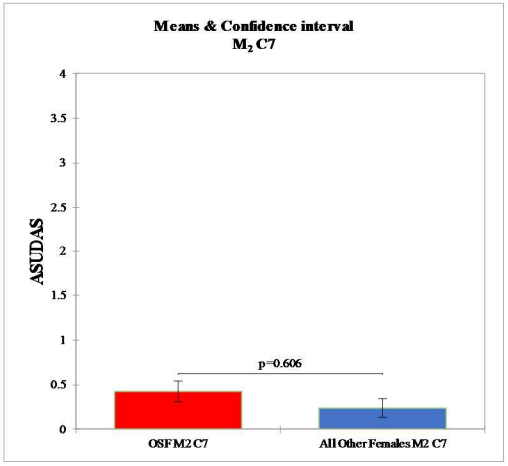
Mann-Whitney test / Two-tailed test:

U	1249
Expected value	1020.000
Variance (U)	10362.125
p-value (Two-tailed)	**<0.0001**
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

Figure 42: Permanent M_2 C7





Mann-Whitney test / Two-tailed test:

U	393
Expected value	343.000
Variance (U)	2364.093
p-value (Two-tailed	0.606
alpha	0.050

The p-value has been computed using 10000 Monte Carlo simulations.

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Supplemental Materials

Each OS individual trait score is averaged by grade score total divided by the number of observations expressed as a percent and an expression of 50% or greater is highlighted.

Table S1: All Traits Male vs. Female

	% OSM Deciduous	% OSF Deciduous
1	43.8%	50.0%
2	50.0%	50.0%
3	43.8%	37.5%
4	35.3%	50.0%
5	53.3%	31.3%
6	41.7%	41.7%
7	29.4%	50.0%
8	35.3%	53.8%
9	50.0%	60.0%
10	52.9%	43.8%
11	47.1%	46.7%
12	42.9%	44.4%

Table S2: All Traits Male vs. Female

Twin pair	% OSM Permanent	% OSF Permanent
1	39.1%	42.1%
2	40.9%	47.6%
3	43.5%	38.1%
4	40.0%	38.1%
5	54.5%	54.5%
6	25.0%	58.3%
7	50.0%	58.3%
8	66.7%	54.5%
9	38.5%	38.5%
10	55.6%	44.0%
11	36.0%	50.0%
12	52.9%	50.0%

Table S3: Canine vs. Molar Deciduous

Twin pair	% Canine Male Deciduous	% Canine Female Deciduous
1	50.0%	50.0%
2	75.0%	75.0%
3	33.3%	25.0%
4	0.0%	25.0%
5	100.0%	75.0%
6		
7	0.0%	50.0%
8	75.0%	75.0%
9		
10	50.0%	75.0%
11	50.0%	50.0%
12		50.0%

Table S4: Canine vs. Molar Permanent

Twin pair	% Male Permanent molar	% Female Permanent molar
1	50.0%	46.2%
2	50.0%	46.2%
3	53.8%	46.2%
4	46.2%	61.5%
5	50.0%	38.5%
6	41.7%	41.7%
7	38.5%	46.2%
8	38.5%	50.0%
9	50.0%	60.0%
10	53.8%	46.2%
11	53.8%	50.0%
12	50.0%	50.0%