ANGLIA RUSKIN UNIVERSITY

FACULTY OF SCIENCE AND ENGINEERING

SCENT-MARKING BEHAVIOUR AND SEMIOCHEMISTRY IN THE CALLITRICHIDAE

ALICE C. POIRIER

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of Anglia Ruskin University for the degree of
Doctor of Philosophy

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À mes cousines et cousins,

Les grands et les petits,

Les proches et les lointains,

Vous êtes ma plus grande force.

Acknowledgements

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Abstract

Olfactory communication is an important mediator of social interactions in mammals, providing information about an individual's identity and current social, reproductive, and health status. Callitrichids (i.e. marmosets and tamarins) constitute a good model for the study of olfactory communication, as they make use of a range of odour signals. Callitrichids conspicuously deposit odorous secretions, produced by specialized scent-glands, on branches in their environment, a behaviour called scent-marking. Several functions have been attributed to callitrichid scent-marking behaviour, including advertisement of reproductive and dominance status, and of identity, territorial defence, and spatial orientation and signalling of food resource location.

The present doctoral project combined behavioural and chemical information to investigate callitrichid olfactory communication. The study explored how environmental, social, and reproductive aspects might influence patterns of callitrichid scent-marking behaviour, as well as the chemical composition of scent-gland secretions and urine used to convey chemosignals. Behavioural observations, along with swabs of scent-glands, and of naturally deposited scent-marks and urine, were collected from captive groups of bearded emperor tamarins, *Saguinus imperator subgrisescens*, cotton-top tamarins, *Saguinus oedipus*, and silvery marmosets, *Mico argentatus*, in three British zoos. Chemical samples were analysed using headspace gas chromatography-mass spectrometry (GC-MS). In addition, scent-gland secretion samples were collected from a wild population of sympatric emperor tamarins and Weddell's saddleback tamarins, *Leontocebus weddelli*, during an annual capture-and-release programme in the south-eastern Peruvian Amazon. These samples were analysed using both *in situ* and laboratory-based GC-MS techniques.

I established the existence of unique chemical signatures of species, groups, sex, reproductive status, and the individual, in callitrichid scent samples, which were matched with differences in scent-marking behaviour. My results support the assumption that chemosignalling plays an important role in the advertisement of identity, reproductive state/status and dominance in this taxon. Moreover, I showed that the social context, as well as spatiotemporal aspects of scent-marking deposition, influenced scent-marking activity. Further differences in the characteristics of scent-marking deposition revealed in this study, both at behavioural and chemical levels, may reflect variable strategies of communication to ensure that signals are transmitted to the intended receivers, which is especially relevant for sympatric species.

I identified a number of putative semiochemicals (i.e. chemicals involved in communication) from the scent samples of captive and wild

callitrichids. Notably, I presented results from the first use of the Torion® portable GC-MS for *in situ* analysis of wild mammal scent samples. In addition, I revealed differences in the chemical composition of tamarin scent-gland secretion samples between wild and captive conditions, which may indicate an effect of captivity on the chemicals produced. This study provides knowledge of mammalian olfactory communication systems, applicable to captive husbandry practices, including conservation breeding programmes of rare species.

Key words: Olfactory communication; primates; gas chromatography–mass spectrometry; chemical signatures; semiochemicals.

Résumé

La communication olfactive est un important médiateur d'interactions sociales chez les mammifères, renseignant sur l'identité d'un individu et son statut social, reproducteur, ou encore de santé. Les callitrichidés (les tamarins et ouistitis) constituent un bon modèle pour l'étude de la communication olfactive car ils font l'usage de nombreux signaux chimiques capables de réguler d'importantes fonctions comportementales. Les callitrichidés déposent des sécrétions odorantes produites par des glandes odorifères spécialisées, de manière visible sur des branches dans leur environnement. Ce comportement se nomme marquage olfactif. Plusieurs fonctions ont été attribuées au comportement de marquage olfactif des callitrichidés, notamment l'avertissement d'identité, de statut reproducteur et de dominance, la défense territoriale, ainsi que l'orientation dans l'espace et la signalisation de ressources alimentaires.

Le présent travail doctoral combine des informations comportementales et chimiques afin d'examiner certains aspects de la communication olfactive chez les callitrichidés. Mon étude explore comment les aspects environnementaux, sociaux, et reproducteurs, peuvent influencer le comportement de marquage olfactif des callitrichidés, ainsi que la composition chimique des sécrétions glandulaires et de l'urine utilisées pour transmettre les signaux chimiques. Des observations comportementales en captivité, accompagnées de prélèvements de sécrétions odorantes et d'urine, ont été collectées sur des groupes de tamarins empereur à barbe (*Saguinus imperator subgrisescens*), de tamarins à crête blanche (*Saguinus oedipus*) et de ouistitis argentés (*Mico argentatus*) dans trois zoos britanniques. Les prélèvements odorants ont été analysés chimiquement par chromatographie en phase gazeuse-

spectrométrie de masse (GC-MS). De plus, des échantillons de sécrétions de glandes odorifères ont été prélevés sur une population sympatrique à l'état sauvage de tamarins empereurs et de tamarins à selle de Weddell (*Leontocebus weddelli*) durant un programme annuel de marquage-recapture en Amazonie péruvienne. Ces échantillons ont été analysés par deux méthodes de GC-MS, *in situ* et en laboratoire.

Durant ce travail, j'ai établi l'existence de signatures chimiques dans les échantillons odorants de callitrichidés, distinguant les espèces, groupes, sexes, statuts reproducteurs, et individus, qui coïncident avec des différences de comportement de marquage olfactif. Mes résultats soutiennent l'hypothèse selon laquelle la signalisation chimique joue un rôle important dans l'avertissement de l'identité, du statut reproducteur et de dominance chez ce taxon. Par ailleurs, j'ai montré que le contexte social, ainsi que les aspects spatiotemporels de la déposition de signaux olfactifs, influençaient l'activité de marquage. J'ai de plus observé d'autres différences dans les caractéristiques du marquage olfactif tant comportementales comme chimiques. Ces différences peuvent refléter des stratégies variables de communication afin de s'assurer que les signaux sont transmis aux receveurs désirés, un mécanisme important notamment chez les espèces sympatriques.

J'ai identifié un nombre de probables substances sémiochimiques (substances chimiques impliquées dans la communication) trouvées dans les échantillons odorants de callitrichidés sauvages et en captivité. En particulier, j'ai présenté les résultats de la première utilisation du Torion® GC-MS, un instrument de GC-MS portable, pour l'analyse *in situ* d'échantillons d'odeurs de mammifères à l'état sauvage. De plus, j'ai révélé des différences dans la composition chimique de sécrétions de glandes odorifères entre tamarins en captivité et à l'état sauvage, ce qui peut indiquer un effet de la vie en captivité sur les substances sémiochimiques produites. Mon étude contribue à une plus grande connaissance des systèmes de communication olfactive chez les mammifères. Elle peut s'appliquer aux procédés d'élevage en captivité, y compris au développement de programmes d'élevage conservatoire pour les espèces menacées.

Mots-clés : Communication olfactive ; primates ; chromatographie en phase gazeuse-spectrométrie de masse ; signatures olfactives ; substances sémiochimiques.

Resumen

La comunicación olfativa es un componente importante en las interacciones sociales de los mamíferos, informando sobre la identidad de un individuo, su estatus social, estado reproductivo o de salud. Los calitrícidos (pichicos o titís) constituyen un buen modelo para el estudio de la comunicación olfativa ya que usan numerosas señales químicas capaces de regular importantes funciones del comportamiento. Los calitrícidos depositan secreciones odorantes producidas por unas glándulas odoríferas especializadas, de manera visible encima de las ramas a su alrededor. Este comportamiento se llama marcaje olfativo. Varias funciones fueron asignadas al comportamiento de marcaje olfativo de los calitrícidos, en particular: la advertencia de identidad, de estado reproductivo y de dominancia; la defensa territorial así como la orientación en el espacio y la señalización de recursos alimenticios.

El presente proyecto de doctorado combina información conductual y química con el fin de investigar algunos aspectos de la comunicación olfativa de los calitrícidos. Mi estudio explora como los aspectos ambientales, sociales y reproductivos pueden influenciar el comportamiento de marcaje olfativo de los calitrícidos, así como la composición química de las secreciones glandulares y de la orina, utilizadas para transmitir las señales químicas. Observaciones de comportamiento en cautiverio, acompañadas de muestras de secreciones odorantes y de orina, fueron recolectadas en tres parques zoológicos británicos. Las especies estudiadas fueron: *Saguinus imperator subgrisescens* (pichicos emperadores o titís bigotudos), *Saguinus oedipus* (titís cabeza de algodón) y *Mico argentatus* (titís plateados). Las muestras odorantes fueron analizadas químicamente por cromatografía de gases-espectrometría de masas (GC-MS). De igual modo, muestras de secreciones de glándulas odoríferas fueron extraídas en una población simpátrica silvestre de *S. i. subgrisescens* y de *Leontocebus weddelli* (pichicos comunes) durante un programa anual de marcado-y-recaptura en la Amazonia peruana. Esas muestras se analizaron por dos métodos de GC-MS, *in situ* y en el laboratorio.

En las muestras odorantes de calitrícidos he establecido la existencia de firmas químicas únicas de especie, grupo, sexo, estado reproductivo y del individuo, las cuales coincidían con diferencias de comportamiento de marcaje olfativo. Mis resultados sostienen la siguiente hipótesis: la señalización química juega un papel importante en la advertencia de identidad, de estado reproductivo y de dominancia en este taxón. Además, mostré que el contexto social así como los aspectos espaciotemporales de la deposición de señales olfativas, influenciaban la actividad de marcaje. Encontré otras diferencias en las características de marcaje olfativo a nivel de comportamiento y a nivel de composición química. Esas diferencias pueden reflejar estrategias variables de comunicación para asegurarse que las señales son transmitidas a los

recipientes esperados, un mecanismo importante sobre todo en especies simpátricas.

Identifiqué varias probables substancias semioquímicas (substancias químicas implicadas en la comunicación) en las muestras odorantes de calitrícidos silvestres y cautivos. En particular, presenté los resultados del primer uso del Torion® GC-MS, un instrumento de GC-MS portátil utilizado en el análisis *in situ* de muestras de olores de mamíferos silvestres. Asimismo, revelé diferencias en la composición química de secreciones de glándulas odoríferas entre pichicos cautivos y silvestres. Estas diferencias indican un efecto de la cautividad sobre las substancias semioquímicas producidas por los animales. Mi estudio contribuye a un mayor conocimiento de los sistemas de comunicación olfativa en los mamíferos, pudiéndose aplicar a los manejos de cría de animales y como herramienta de conservación de especies amenazadas, desarrollando programas de cría en cautividad.

Palabras claves: Comunicación olfativa; primates; cromatografía de gases-espectrometría de masas; firmas olfativas; substancias semioquímicas.

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Glossary[1]

Aliphatic compound – An open-chained molecule, either straight or branched, or a cyclic non-aromatic compound.

Aromatic compound – An unsaturated, planar, cyclic molecule containing conjugated double bonds or electron pairs with a total of 4n+2 π electrons: most commonly n=1.

Behavioural bioassay – The study of an animal's specific behavioural and/or physiological response to an odour signal.

Callitrichidae – The family of New World primates, composed of the tamarins (*Saguinus* spp. and *Leontocebus*), the lion tamarins (*Leontopithecus* spp.), the marmosets (*Mico* and *Callithrix* spp.), the pygmy marmosets (*Cebuella* spp.), and the Goeldi's monkey, *Callimico goeldii*.

Catarrhines – The group of the Old World primates and apes, characterized by nostrils close together and opening downwards, and a non-prehensile, often greatly reduced or vestigial tail.

Chemical diversity – The combination of individual compounds in a scent sample.

Chemical richness – The number of compounds in a scent sample.

Chemosignalling – The communication by the means of chemical signals, i.e. **chemosignals**.

Chromatogram – The pattern of separated compounds obtained by chromatography.

Flehmen – The behavioural response of many animals to chemical signals, consisting of lip curling, head raising, and a long inhalation with the nostrils usually closed, which facilitates the transfer of volatile chemicals into the vomeronasal organ.

Headspace – The volume above a liquid or solid in a closed container.

Ionization (in GC-MS) – The formation of ions by adding or removing electrons from atoms, for instance by action of a highly energetic electron field inside a mass spectrometer.

Kairomone (*sensu* Wyatt, 2014a) – A chemical signal liberated by prey, used by predators.

[1] Some of the definitions given in this glossary may be adapted to the specific use of these terms for the present thesis.

Macrosmatic / Microsmatic – Having a good/bad sense of smell.

Mass spectrum (in GC-MS) – The pattern of relative abundance of fragment ions of different mass-to-charge ratio derived from a compound coming out of the mass spectrometer.

Mobile phase (in GC-MS) – a flow of inert gas (often helium, more rarely nitrogen or hydrogen), circulating inside the gas chromatograph and carrying the mixture of compounds being analysed.

NIST mass spectral library (in GC-MS) – A database, developed and supported by the National Institute of Standards and Technology, of mass spectra of known molecules, used to compare with the mass spectra of unknown compounds and suggest the best match as putative identity.

Pheromone (*sensu* Wyatt, 2014a) – A chemical signal used for intraspecific communication.

Pheromonatherapy – The use of chemical signals to manage stress-related behavioural disorders in pets.

Platyrrhines – The group of the New World primates, characterized by nostrils far apart and opening forwards or sideways, and often a prehensile tail.

Primer effect – A long-lasting physiological or developmental change, sometimes mediated by hormones, to a chemical signal.

Releaser effect – An immediate behavioural response to a chemical signal.

Scent-gland – A specialized secretory gland producing an odorous substance used for scent-marking by many mammals. Location, size and histology of scent-glands vary across species. Examples of scent-glands in this study are the anogenital gland, the suprapubic gland, and the sternal gland.

Scent-marking – The marking of a substrate with an odorous substance secreted from a specialized scent-gland.

Semiochemical – A chemical emitted by an organism that influences the physiology or behaviour of an organism of the same or a different species.

Semiochemistry – The study of the chemical means of communication used by living species.

Stationary phase (in GC-MS) – An absorbent polymer coated into the inside of a long column in the gas chromatograph, of varying chemical affinity for the compounds composing the sample being analysed.

Strepsirrhines – The primate suborder including the lemurs, lorises, galagos and pottos, characterized by a moist area around the nostrils.

Synomone (*sensu* Wyatt, 2014a) – A mutualistic chemical signal, recognized by more than one species.

Unsaturated compound – A straight or cyclic molecule containing one or more carbon-carbon double or triple bonds. Many natural organic compounds are unsaturated.

Vomeronasal organ (or Jacobson's organ) – A pair of parallel tubules situated on either side of the nasal septum of the buccal cavity involved in chemoreception, found in reptiles, amphibians, and most mammals.

Copyright declaration

I declare that this thesis, and the research that it details, are the result of my own work. I acknowledge the helpful guidance of my supervisors, Dr Andrew Smith, Professor John Waterhouse, and Dr Jacob Dunn, and the useful collaboration with Field Projects International and Dr Amanda Melin for the section of my work carried out in wild conditions. I confirm that where the work of others was used to inform my research, I provided explicit citations. I further state that no part of my work has been submitted for any other qualification, either at Anglia Ruskin University, or at any other institution.

Word count: 55,970

Signed  Alice Caroline Poirier

Chapter I – General introduction and study outline

I.1. Importance of chemical communication in the animal kingdom

I.1.1. Definitions

Chemical communication is the oldest and most diverse form of communication, shared by all organisms including bacteria; all animals are pre-adapted to detect chemical signals – chemosignals – in their environment (Wilson, 1970; in Wyatt, 2014a). Chemical information in animals is used in territorial defence and resource marking, as well as to exchange signals during social interactions (Müller-Schwarze, 2006). In a large number of animal species, chemosignals of an incredible diversity have evolved to become an important mediator of social and sexual interactions (Wyatt, 2014a). This is especially true in mammalian species, which are often particularly social animals, using multiple modes of communication to exchange information among conspecifics. Mammalian social systems hinge upon acoustic, visual, facial, and olfactory signals that convey information between individuals, both intra- and inter-specifics (Arlet et al., 2015; Liebal et al., 2014; Partan, 2013). Olfactory communication often involves complex chemosignals, which can give conspecifics information on identity, i.e. at the levels of species, group, kinship, sex, and the individual (Brennan and Kendrick, 2006; Johnston, 2003; Wyatt, 2014a), and current status, i.e. social, reproductive, and health status (Brown and Macdonald, 1985; Drea, 2015; Wyatt, 2014a).

Chemosignalling has been subject of investigation in the fields of animal communication, ecology, and reproduction, for a long time (e.g. Cheal and Sprott, 1971; Ritter, 1979; Thiessen et al., 1976), notably since Karlson and Butenandt's key stone discovery of moth sexual chemical signals (Karlson and Butenandt, 1959). Nevertheless, progress in the understanding of mammalian chemosignalling has been slow in comparison with other communication modalities, mainly due to methodological constraints (Albone and Shirley, 1984; Müller-Schwarze,

2006). Only recently has the field of semiochemistry, i.e. the study of chemical means of communication used by living species (Albone and Shirley, 1984), emerged as a key point of study for mammalian taxa (**Fig. I.1**; see **Appendix A**, **Table A.1**). Research in this field has been greatly enhanced by the rapid development of analytical chemistry techniques in the early 2000s (see section I.2.2; Apps, 2013; Soso et al., 2014), thus providing an exciting prospect for ongoing research on the subject. This is especially true for the primate order, including humans, in a continuous effort to shed light on the evolution of humanity, and further justifies the present doctorate project.

The current literature uses various terms to define the different types of animal chemosignals, well described in Wyatt (2014a). Notably, pheromones (Karlson and Lüsher, 1959) are chemosignals used for intraspecific communication, for instance in intrasexual competition and mate choice; kairomones are prey signals used by predators; and synomones are mutualistic signals. However, the exact definitions of the various types of chemosignals are still debated; therefore I have decided to only use generic terms in my study, such as chemosignals, signalling compounds, semiochemicals, olfactory signals, and odour signals, a conservative choice made by many other authors in the field of semiochemistry (e.g. Alberts, 1992; Apps, Weldon and Kramer, 2015; Snowdon et al., 2006).

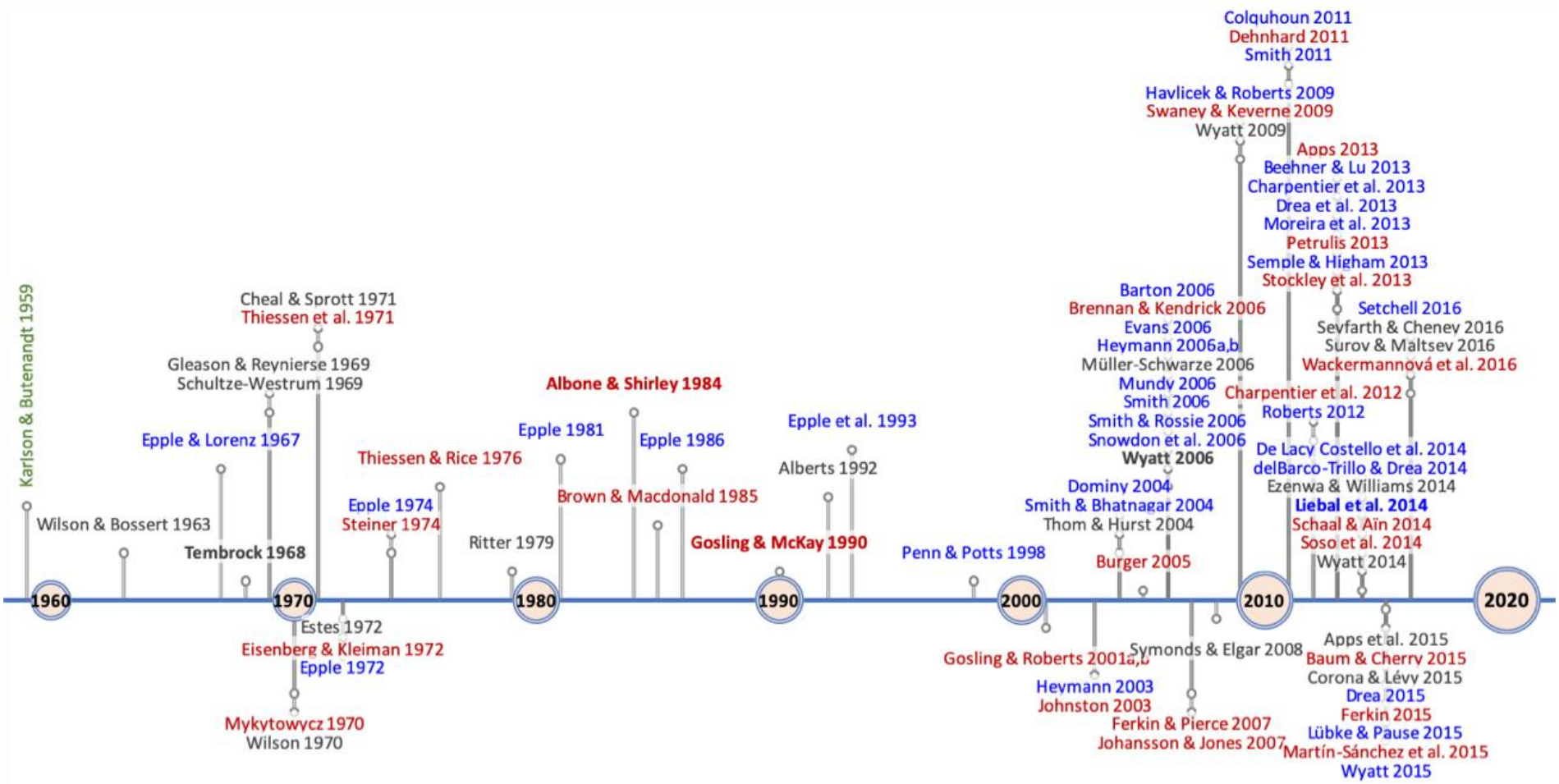


Figure I.1. History of research on mammalian semiochemistry before the start of the current project, showing the acceleration of published work in this field after 2000, as analytical techniques improved. Karlson & Butenandt's keystone publication on moth pheromone is shown in green; the rest are review publications, and books (in bold), on ***i.*** vertebrates (in black), ***ii.*** mammals (in red), and ***iii.*** primates (in blue), on the exclusive or partial topic of mammalian semiochemistry (see **Appendix A**, **Table A.1**). Original experimental or observational published work is not included.

I.1.2. Chemical communication pathways in mammalian species

I.1.2.1. Signal production

Mammalian chemosignals are complex mixtures of volatile and non-volatile compounds. Properties of these chemosignals depend upon when, where and how they were emitted, the compounds assemblage, as well as the physical properties (e.g. volatility) and chemical properties (e.g. functional groups) of the component compounds (Müller-Schwarze, 2006). These compounds may be direct by-products of essential biochemical pathways, derived from the environment (e.g. through diet), or produced by commensal bacteria (Archie and Theis, 2011; Ezenwa and Williams, 2014). They may be passively conveyed in body fluids and excretions, or actively produced in glandular secretions.

Glandular secretions are fluids produced by different types of exocrine glands, the apocrine sweat glands, the sebaceous glands producing a lipid secretion, and specialized glands such as the mammary glands producing milk, and the anal glands of carnivores (Burger, 2005). A range of specialized secretory glands, called scent-glands, have a specific role in chemosignalling. Scent-gland secretions are conspicuously deposited in the environment (e.g. on rocks or branches) as a form of chemical message, a behaviour called scent-marking. Sometimes, the scent signal is produced by mixing several body fluids together, such as urine, vaginal/seminal discharge, and anogenital scent-gland secretion (e.g. in wolves, *Canis lupus*, Asa et al., 1985; and female giant pandas, *Ailuropoda melanoleuca*, Hagey and Macdonald, 2003). This demonstrates the variety and complexity of the chemical cues mediating social communication.

I.1.2.2. Chemoreception and signal integration

Chemosignals are recognized and processed by the recipient, which is often a conspecific individual, and sometimes a very different organism, such as in prey–predator recognition (Saavedra and Amo, 2018; Wyatt, 2014a). The volatile components in secretions evaporate after deposition, and signals become weaker over time. Evaporation of the more volatile compounds may change the quality of the odour, as well as the

range over which it can be detected. Nevertheless, such decay and change in the signal may also provide information about the age of the scent-mark and, in turn, the recent behavioural history of the signaller (Müller-Schwarze, 2006).

Chemoreception is performed by two main chemosensory systems in mammals: the main olfactory system (MOS), and the accessory olfactory system (AOS), well described in Müller-Schwarze (2006). The MOS is formed by the main olfactory epithelium (MOE) beneath the nose, and its corresponding main olfactory bulb (MOB) in the brain (**Fig. I.2a**); the AOS is composed of the vomeronasal organ (VNO), also called Jacobson's organ, located above the palate on either side of the nasal septum, and its corresponding accessory olfactory bulb (AOB; **Fig. I.2b**).

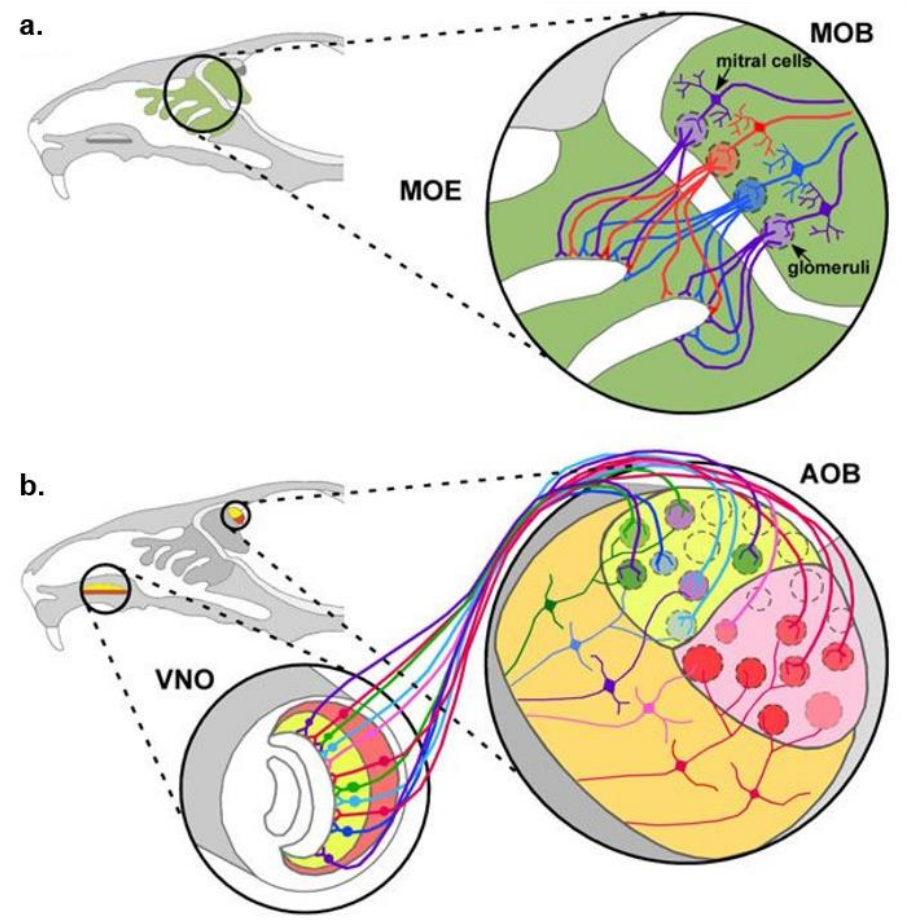


Figure I.2. Schematic diagrams of the mouse **a.** main, and **b.** accessory olfactory systems. MOE: main olfactory epithelium; MOB: main olfactory bulb; VNO: vomeronasal organ; and AOB: accessory olfactory bulb (reproduced from Dulac and Wagner, 2006).

The main components of the MOE are the olfactory sensory neurons (OSNs), connecting the mucosa covering the nasal cavity with the glomeruli in the frontal part of the brain (**Fig. I.3**). The nasal mucosa contains specific odorant-binding proteins that retain the volatile compounds present in odours. OSN dendrites have cilia floating in the mucosa, covered in binding receptors specific to a certain type of odorant compound. When an odorant compound binds with the OSN, the signal is transduced along the axon, and delivered in the corresponding glomerulus of the MOB. Information is then processed in the brain at higher centres. There are many different OSN types, all encoded in the olfactory receptor genes, which constitute the largest multigene family in mammals (Issel-tamer and Rine, 1997).

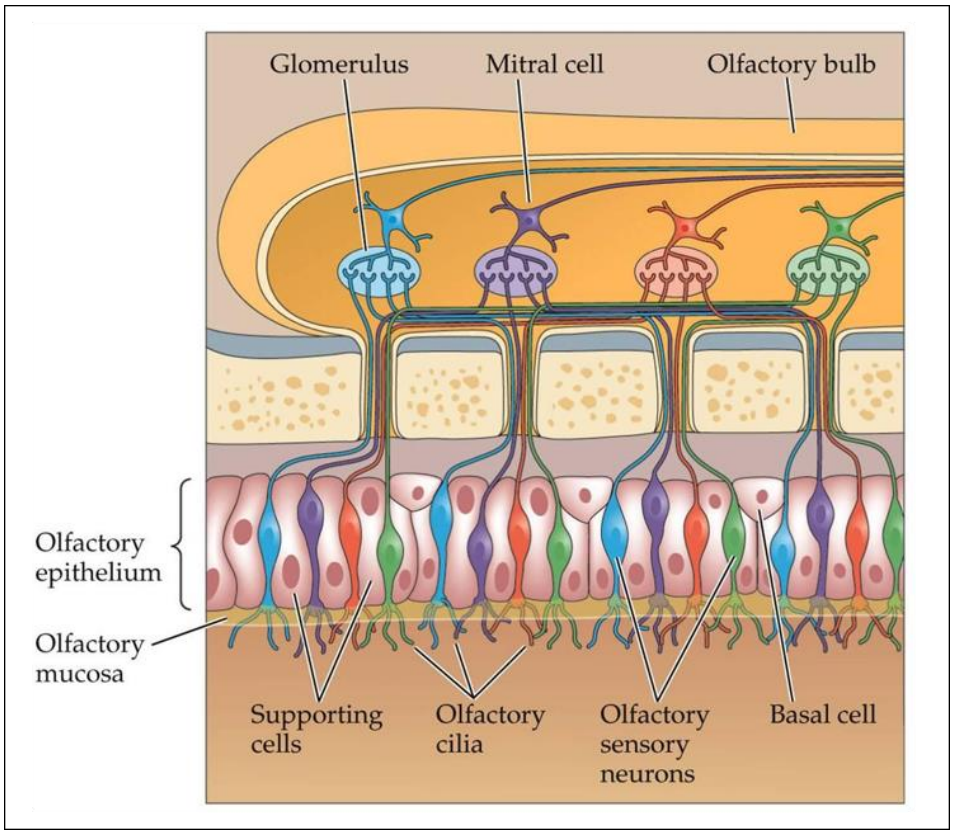


Figure I.3. Schematic diagram of a transversal section of the mammalian main olfactory system (reproduced from Wolfe et al., 2017).

Although most mammals possess both MOS and AOS, there is currently a debate over the functionality of the VNO in some species formerly considered to have a poor sense of smell, such as Old World primates including apes (Evans, 2006; Baum and Cherry, 2015;

Charpentier et al., 2013). Unlike the MOE receptors, which can only bind with volatile odorant compounds, the AOS receptors capture signals from both volatile and non-volatile compounds, such as lipids and proteins, contained in fluids or solids that can be swallowed. Yet MOS and AOS partially overlap in function, the extent of which differs between taxa (Mucignat-Caretta, Redaelli and Caretta, 2012). The VNO is formed of a pair of parallel tubules, lined with OSNs, which axons connect to the AOB (**Fig. I.2b**). A well-known behaviour related to the VNO activity is flehmen (Schneider, 1930), in which an animal opens its mouth with its lips curled in a 'laughing display', in the direction of an scent source, to pick up odours in its mouth (**Fig. I.4**). Mammals are also known to sometimes lick or muzzle-rub on deposited secretions or urine of congeners for this same physiological reason (reviewed in Estes, 1972).

Figure I.4. Flehmen behaviour in the plains zebra, *Equus quagga* (from www.commons.wikipedia.org).

Although there is a lot of research conducted on the neurophysiology of odour detection and recognition in mammals (e.g. Barkai and Wilson, 2014), it is not the focus of the present thesis. I will instead concentrate on the proximate functions of chemical communication in the social and sexual behaviours of mammals, in particular primates.

I.2. Methodological approaches to the study of mammalian chemosignalling

In mammals, olfactory communication is both chemical and behavioural. Chemosignals produced by the signaller are liberated in the environment, either via passive exudation of body odours or in excretions, or via active deposition of scent-gland secretions during scent-marking. Some mammalian species also perform urine-marking, where urine and/or faeces, sometimes mixed with glandular secretions, is applied on a substrate, usually the vertical face of a rock, or a prominent log or mount (Apps, 2013). In primates in particular, scent-marking is often performed in front of conspecifics (Laska and Hudson, 1995; Lazaro-Perea, 2001), which suggests that the visual cue associated with scent deposition is also important. This conspicuous behaviour has instigated numerous studies in captive, semi-free range, and wild conditions (see section I.3.2).

I.2.1. Behavioural approaches

I.2.1.1. Behavioural observations: study of scent-marking behaviour

During behavioural observations, social and ecological information may be recorded, together with scent-marking patterns, such as scent-marking frequency according to sex, social or reproductive status, and the spatial distribution of scent-marks, in order to infer the likely functions of scent-marking behaviour. Three functional hypotheses are commonly considered: **1.** spatial orientation and signalling of food resource location; **2.** territorial advertisement and defence; and **3.** regulation of social and reproductive dominance, as well as intrasexual competition/intersexual mate choice (Apps, Weldon and Kramer, 2015; Heymann, 2006a; Seyfarth and Cheney, 2016; Wyatt, 2014a).

I.2.1.2. Behavioural bioassays: study of specific responses to odour signals

In the case of behavioural bioassays applied to olfactory communication, the proximate functions of olfactory signals on the receiver are inferred from observations, usually in experimental conditions (Haynes and Millar, 1998). Typically in a behavioural bioassay, an odour, either

artificially synthetized, or sampled from a congener or another animal or plant, is presented to the target individual, and its response, behavioural and/or physiological, is recorded (Thom and Hurst, 2004). Experimental controlled conditions in captive breeding centres and medical research facilities allow the isolation of precise signal triggers and responses, which is very valuable for trying to unravel the mechanisms and functions of chemosignal transmission. For example, Swaisgood et al. (2000) found evidence for the discrimination of sex and female reproductive condition via olfactory cues in captive male giant pandas. Similarly, Smith and Abbott (1998) showed that common marmosets, *Callithrix jacchus*, can discriminate between circumgenital scent-marks from periovulatory and anovulatory females. Moreover, Henkel and Setchell (2018) suggested that chimpanzees, *Pan troglodytes*, recognize group members and kin via olfactory cues in urine; and Scordato and Drea (2007) showed that male ring-tailed lemurs, *Lemur catta*, respond primarily to odours from breeding and dominant individuals, compared with subordinate congeners. However, in wild conditions such behavioural bioassays can be very challenging to put in place, and many more factors are likely at play in natural conditions (Charpentier et al., 2012; Drea et al., 2013).

I.2.2. Semiochemical approaches

I.2.2.1. Principles of semiochemical analysis of animal scents

Semiochemical analyses use analytical chemistry techniques to describe the chemical composition of animal scents used as signals. Particular organic compounds may serve as chemosignals in various animal species, regardless of whether the ‘chemical meaning’ of a single compound is the same across species. To use an analogy, we can relate organic compounds to individual letters, which when associated together form various chemical messages (i.e. ‘words’) and can be used across different species (i.e. ‘languages’). Moreover, mammalian chemosignals may be either single compounds or mixtures, and are as likely to be coded by the presence and absence of compounds, as by their relative concentration, adding a layer of complexity in research in this field (Apps, 2013).

In their recent review, Soso and co-workers (2014) identify the most

widely used analytical methods for chemical and sensory characterization of scent-marking in large wild mammals. They describe the methods currently used for the study of mammals, from New World primates to large felines, with field applications. Starting from Soso et al.'s review, I present the main techniques for sampling and sample extraction, chemical analysis, and data analysis, followed by an assessment of potential applications to the current project.

I.2.2.2. Scent sampling and sample extraction

I.2.2.2.1. Scent sample collection and storage

Scent samples are usually collected on cotton or viscose swabs, previously washed in an organic solvent (methanol and/or pentane), by rubbing the substrate after natural scent-mark deposition, or the scent-gland skin area from anaesthetized or restrained animals. Naturally deposited scent-marks can sometimes be collected on filter paper placed in the animals' environment (e.g. in captive mandrills, *Mandrillus sphinx*, Vaglio et al., 2016). Captive animals, particularly primates, can also be trained to deposit their scent-marks on glass plates or tubes tied to a perch (e.g. common marmosets, Smith et al., 1997). Urine is easier to collect, as it is usually produced in a more conspicuous quantity than scent-mark depositions.

Generally, samples are immediately stored in solvent-washed glass vials and kept frozen in order to avoid sample decay. Contamination can be limited by using storage vials directly usable for analysis (e.g. glass chromatography vials with septum-fitted lids). Vials are often kept frozen until analysis, at -20°C to -80°C, for as long as six months (Birkemeyer et al., 2016; Drea et al., 2013). Freezing seems to have no effect on sample composition and perceived odour (Lenochova, Roberts and Havlicek, 2009).

I.2.2.2.2. Solvent-based sample extraction technique

With solvent-based extraction technique, an organic solvent is used to dissolve the volatile compounds present in the sample. Solvents commonly employed are methanol- and ethanol-based, sometimes mixed with water. Usually, the use of solvent-based extraction involves two to

three steps (Drea et al., 2013): **1.** dissolving the volatile compounds into the solvent; **2.** concentrating the compounds by evaporating part of the solvent; and **3.** (optional) applying chemical derivatization in order to increase the volatility of the compounds, and hence reduce their processing time.

Solvent-based extraction is often used for the identification of peptides and proteins, which are of particularly high polarity and low volatility. This technique is very popular in most of the literature on mammalian semiochemistry and is particularly cost-efficient. However, several disadvantages of solvent-based extraction are pointed out by Soso et al. (2014): they require a series of procedures and are time-consuming; moreover, they can affect the chemical composition of a sample due to interactions between the compounds and the solvent, or solvent impurities. In addition, solvents are harmful for the environment and must be disposed of in a safe manner, thus complicating the procedure. This method is currently widely used for the analysis of primate scent-marking, notably by Drea and co-workers who are leading the field of olfaction and behaviour in captive strepsirrhines (Boulet, Charpentier and Drea, 2009; Charpentier et al., 2008; Crawford, Boulet and Drea, 2011; Greene and Drea, 2014; Scordato, Dubay and Drea, 2007).

I.2.2.2.3. Solvent-free sample extraction techniques

In recent years, several solvent-free, environmentally benign, sampling techniques have been developed. These solvent-free techniques directly extract the volatile constituents of the headspace, i.e. the air above the sample, of an enclosed sample. Headspace sample extraction offers the advantages of reducing sample preparation time and simplifying the process for the extraction of volatile compounds, as well as minimising the interferences and impurities induced by solvents. Different solvent-free techniques exist and have been used for the analysis of mammalian semiochemicals: direct headspace extraction, precolumn heater, solid-phase microextraction, stir-bar sorptive extraction, solid-phase dynamic extraction, and thermal desorption, described in **Table I.1**.

Table I.1. Description of solvent-free sample extraction techniques used for the characterization of mammalian odours, including the main processes involved, examples of mammalian studies using these techniques, and an assessment of each technique.

Extraction technique	Main processes involved	References	Assessment (positive: 👍, negative: 👎)
Direct headspace extraction	Volatile compounds from a solid or liquid matrix transferred into the vapour phase and carried by a carrier gas	Volatile compounds in urine of lion, *Panthera leo* (Andersen and Vulpius, 1999)	👎 Older technique, replaced by more effective methods
Precolumn heater	Glass cylinder heated to 100°C with nitrogen being released simultaneously and driving the volatile compounds out of the sample	Volatile compounds in interdigital glands of reindeer, *Rangifer tarandus* (Andersson, Brundin and Andersson, 1979)	👎 Older technique, replaced by more efficient methods
Solid-phase microextraction (SPME)	Use of a fused-silica fibre coated with absorbent thin polymeric film, to passively diffuse volatile compounds from a liquid or solid sample via adsorption, absorption, or capillary condensation	Scent-gland volatile compounds in ring-tailed lemur, *Lemur catta* (Hayes, Morelli and Wright, 2004; Knapp, Robson and Waterhouse, 2006)	👍 Can be used for *in vivo* extraction of volatiles 👍 Various fibre coatings and fibre lengths available to optimize the compounds to be extracted (e.g. from very volatile–semi-volatile, polar–non-polar) 👍 Can be easily automatized 👍 Can be cleaned and reused 👎 Fibre is quite fragile 👎 Static technique, which limits the number of compounds extracted
Stir-bar sorptive extraction (SBSE)	Use of a polymer-coated (usually polydimethylsiloxane [PDMS]) magnetic bar, constantly stirred, to actively extract volatile and semi-volatile compounds from liquid and gaseous samples	Volatile compounds in human body odour (Penn et al., 2007)	👍 Can be easily automatized 👍 Dynamic technique, allows for quantification and extraction of samples of low concentration 👍 Can be cleaned and reused 👎 Expensive 👎 Requires specialized add-ons for analysis and detection of compounds

Table I.1. Continued.

Extraction technique	Main processes involved	References	Assessment (positive: 👍, negative: 👎)
Solid-phase dynamic extraction (SPDE)	Use of repetitive dynamic flow of liquid or gaseous sample components over an absorbent polymer coating (usually PDMS) on the inside wall of a stainless steel syringe needle.	Volatile compounds in urine of brown lemurs, *Eulemur* spp. (DelBarco-Trillo et al., 2011), and African elephant, *Loxodonta africana* (Goodwin et al., 2008)	👍 Dynamic flow permits concentration of volatile compounds 👍 Can be easily automatized 👍 Various types of polymer coating available 👍 SPDE needle more robust than SPME fibre 👍 Can be cleaned and reused 👎 Expensive 👎 Requires specialized add-ons for analysis and detection of compounds
Thermal desorption tube	Gaseous sample is pumped inside a stainless steel tube internally coated with absorbent polymer, which retains the volatile compounds	Volatile compounds in body odour of common marmoset, *Callithrix jacchus* (Kücklich et al., 2017)	👍 Can be used for *in vivo* extraction of volatiles 👍 Tubes can be transported and stored without affecting the quality of the sample they contain 👍 Various types of polymer coating available 👍 Can be cleaned and reused 👎 Expensive 👎 Requires specialized add-ons for analysis and detection of compounds
Electronic nose	Use of the pattern of response of an array of gas sensors to match a known odour pattern	Scent-gland volatile compounds in ring-tailed lemur (Staples and Electronic Sensor Technology, n.d.)	👍 Sample extraction and chemical analysis performed by the same device 👍 Portable 👎 Limited to pre-set 'learnt' odour patterns 👎 No discrimination between individual compounds, only full odour patterns

Thermal desorption, solid-phase microextraction, and solid-phase dynamic extraction are now the most popular solvent-free extraction techniques for the analysis of biological samples, notably because they combine sampling and sample extraction with a procedure that is both simple and efficient (Ramos, 2012; Soso et al., 2014). The main disadvantage in using these methods is that fibres, desorption tubes and needles, used to extract the volatile compounds from the headspace of a sample, are more expensive than organic solvents; but since they can be used multiple times, costs can be optimized.

A novel instrument for direct headspace extraction coupled to real-time chemical analysis, atmospheric chemical ionization-mass spectrometer (APCI-MS; Linforth and Taylor, 1997), was used to monitor real-time production of volatile chemicals by parasitoid wasps. The wasps were placed in air tight chambers were they could behave freely, in the context of female-female competition (in *Goniozus legneri*; Goubault et al., 2006), or female courtship by males (in *Spalangia endius*; Mowles et al., 2013). The air inside the chamber was continuously drawn through a tube inside an APCI-MS detector, which provided real-time occurrence and quantity of the volatile compounds released by individual wasps. Such *in vivo* chemical analyses have not yet been implemented on mammalian species.

Electronic noses can identify an odour using the pattern of response of an array of gas sensors to match a known odour pattern (Nagle, Schiffman and Gutierrez-Osuna, 1998). These devices are notably used in human clinical research, such as for the screening of diseases in body odour and breath (e.g. differentiation of cancerous cells from healthy ones, Kateb et al., 2009; detection of signs of pneumonia infection in the breath, Hanson III and Thaler, 2005; reviewed in Röck, Barsan and Weimar, 2008). Electronic noses have also been employed for the screening of tuberculosis infection in cattle, *Bos taurus*, and European badgers, *Meles meles* (Fend et al., 2005). Moreover, they are often used to characterize odours of plants (Huang et al., 2011) and food (Röck, Barsan and Weimar, 2008) for manufacturing applications. Ongoing research is aiming at widening the range of uses of electronic noses; for example the chemical composition of ring-tailed lemur scent-gland samples was tentatively characterized by an electronic nose (Staples and Electronic Sensor Technology, n.d.).

I.2.2.3. Chemical analysis

I.2.2.3.1. Principles of chemical analysis

Mammalian odours are analysed almost exclusively using gas chromatography (GC), and sometimes high performance liquid chromatography. In GC, a sample is injected into the injection port where it is heated to a vaporous phase, and carried by the mobile phase, i.e. a flow of inert gas (often helium, more rarely nitrogen or hydrogen). This gas flow carries the mixture of compounds into the stationary phase, i.e. an absorbent polymer coated into the inside of a long column (usually 30 m). Inside the gas chromatograph column, the mixture of compounds is separated into individual volatile and semi-volatile compounds according to their relative affinity for the stationary phase, their polarity, and chemical structure, which elute out of the column one after another (**Fig. I.5**). Each compound is therefore characterized by its time of elution, called retention time. GC is ideal for detecting and separating relatively small compounds (<550 daltons), of low polarity and high volatility.

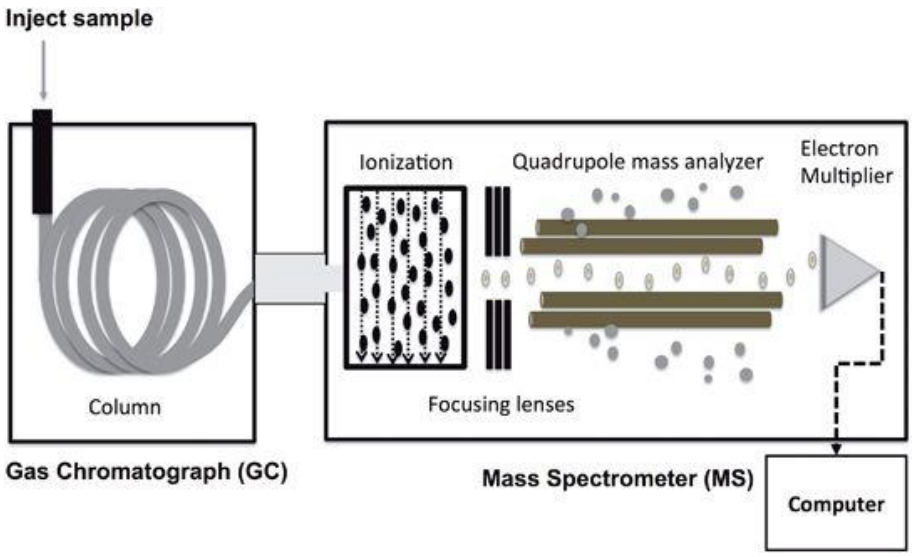


Figure I.5. Schematic diagram of a gas chromatograph-mass spectrometer (reproduced from Kim et al., 2016).

Combined with a detector, GC allows for the detection of individual compounds within a sample. The most commonly used detectors are mass spectrometer, flame ionization detector, and Fourier-Transform infrared spectroscopy. Mass spectrometry (MS) is the most widely used detector because of its capacity to perform a mass spectral search and match for

over 200 000 compounds within its mass spectral library (National Institute of Standards Technology, NIST; Shen et al., 2014; Stein, n.d.; **Fig. I.5**). However, MS is not ideal for the detection of compounds of high molecular weight. Sometimes two detectors can be combined for a better result.

When the compounds elute from the gas chromatograph into the mass spectrometer, they are broken into ionized fragments, which gives them a specific mass-to-charge ratio (m/z). The MS then separates the ions according to their m/z, and records the relative abundance of each ion type (Baker, 2010). Various ion sources can be found in a mass spectrometer, including electron ionization: based on electron excitation, it induces strong fragmentation of the molecules of the compound being analysed. Electron ionization is most commonly used for gaseous samples, hence is often coupled to GC (present study; **Fig. I.5**). Alternatively, chemical ionization produces ions through collision of the compounds with primary ions present in the source, either at high temperature (e.g. investigation of the comb wax of honeybees, *Apis* sp.; Aichholz and Lorbeer, 2000), or at atmospheric temperature (e.g. real-time monitoring of the production of volatile chemicals by female parasitoid wasps; Mowles et al., 2013).

I.2.2.3.2. Result outputs: chromatogram and mass spectra

The primary output of a GC-MS analysis, called a chromatogram, is a graphical representation of the diverse compounds composing a chemical sample, as a function of their time of elution, i.e. retention time (**Fig. I.6a**). Each peak on the chromatogram represents a unique compound (or sometimes a mixture of several compounds), its area the relative abundance of this compound in the sample. Mass spectrometers have different sensitivities for different compounds, depending on their chemical properties. As a result, although peak area may indicate the relative abundance of a same compound across two chromatograms (i.e. from two different samples), the area of different peaks on a same chromatogram are not necessarily in the ratio of their relative concentrations. Each peak is characterized by a mass spectrum, representing the distribution and abundance of ion fragments composing the compound in function of their m/z (**Fig. I.6b**). Mass spectral libraries, such as the NIST library, compare this mass spectrum with those of known compounds in their database, and provide the best matches as putative identity (**Fig. I.6c**).

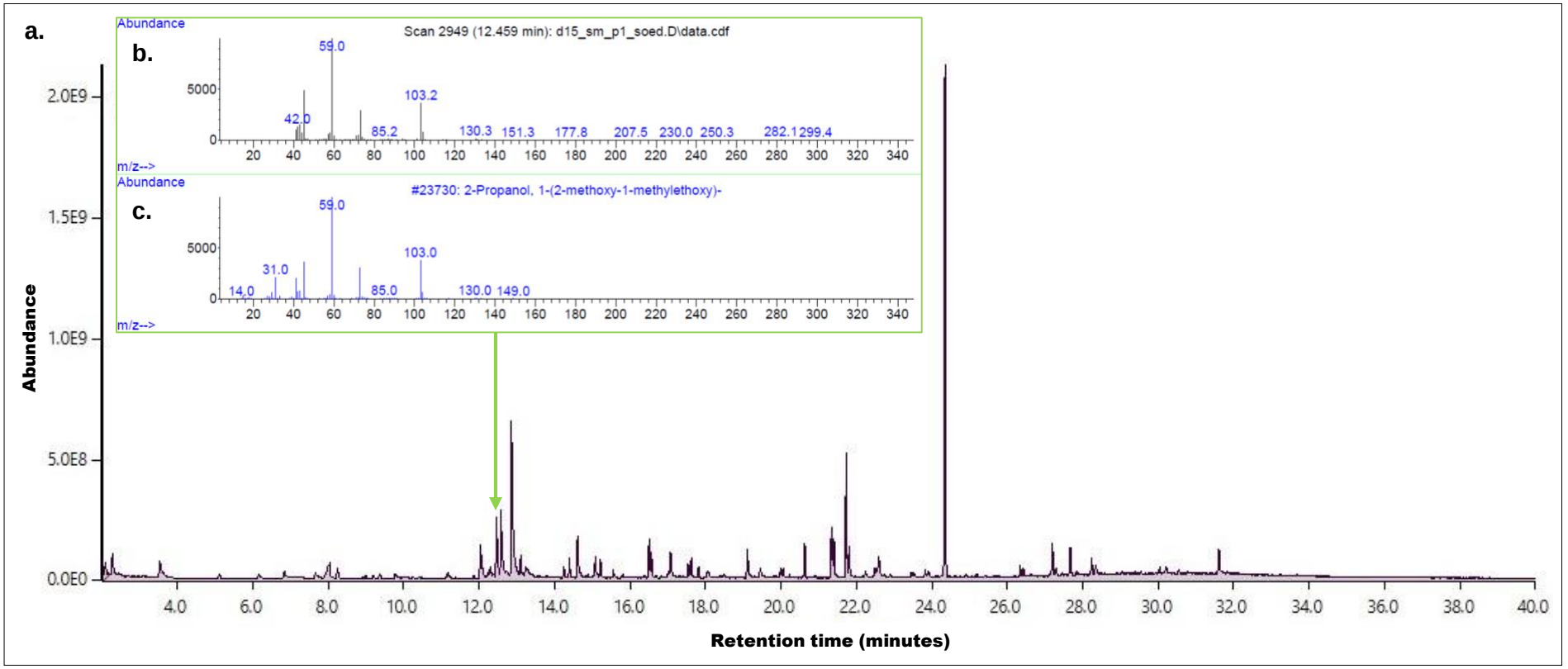


Figure I.6. a. Example of a chromatogram produced by SPME–GC-MS analysis of a scent-mark sample from a female cotton-top tamarin, *Saguinus oedipus*, where each peak represents a unique compound; **b.** Mass spectrum of the peak indicated by a green arrow, showing the relative abundances of ion fragments originating from the molecules of the particular compound, in function of their mass-to-charge ratio (m/z); **c.** Mass spectrum of 1-(2-methoxy-1-methylethoxy)-2-propanol, given as putative identity by the NIST mass spectral library with 83% match.

I.2.3. Endocrinology approaches

When performing behavioural bioassays, the animal's physiological responses to the presentation of an olfactory cue may be recorded alongside the behavioural response. Subtle changes in endocrinal activity in response to olfactory cues may give a more detailed view of the mechanisms triggered inside the recipient's body. The level of hormones involved in behavioural regulation, such as cortisol, oestrogen, progesterone, testosterone, oxytocin, and prolactin, may be measured in samples of urine, faeces, saliva, and hair, all of which can be collected non-invasively (Petrulis, 2013). In particular, a new emphasis is being placed at developing non-invasive endocrinology techniques to monitor hormone–behaviour interactions in wild mammals (Whitten, Brockman and Stavisky, 1998), notably primates (Bales et al., 2006; Higham, 2016). Recent reviews by Petrulis (2013), and Anestis (2010), summarize the relation between chemosignals, hormones, and reproduction and sociality in mammals, and in primates, respectively.

I.3. State of research in primate chemosignalling

I.3.1. History of this field in primatology: olfaction as the neglected sense

Since the beginning of systematic research on the evolution and behavioural ecology of humans and other primates, this taxon was considered to be microsmatic, i.e. to have a poor sense of smell, compared with macrosmatic mammal groups such as carnivores and rodents (Albone and Shirley, 1984; Andersson, 1994). As a result olfactory communication in primates has been understudied (Heymann, 2006b). The consideration of primates being microsmatic was mainly based on the hypothesis of an evolutionary trade-off between vision and olfaction in social species (Barton, 2006; Gilad et al., 2004; Kemp and Kaplan, 2012; Liman and Innan, 2003; Melin et al., 2017), partly related to the comparatively smaller size of the olfaction-related morphological structures in the brain (Le Gros Clark, 1959; Smith and Bhatnagar, 2004; Smith and Rossie, 2006). Moreover, the relatively inconspicuousness of olfactory-related behaviours in primates, compared with acoustic and visual behaviours, and the

methodological difficulties of recording and quantifying odour signals, especially in field conditions, have biased our comprehension of the importance of this mode of communication in this taxon (Epple, 1986; Heymann, 2006b; Wyatt, 2015). Nevertheless, as outlined by Drea (2015) in her recent review, 'D'scent of Man: a comparative survey of primate chemosignalling in relation to sex', parodying Darwin's pioneer publication 'The Descent of Man and Selection Related to Sex' (1871), researchers in the field of semiochemistry are increasingly recognizing the prominent role of olfactory communication in the social and sexual lives of primates. Recently, we have been witnessing advances in the understanding and development of analytical methods for the study of semiochemistry in a range of vertebrate taxa, e.g. in carnivores (Buesching, Waterhouse and Macdonald, 2002a; b; Gilad et al., 2016; Jordan et al., 2010; Soso and Koziel, 2016, 2017; Weiß et al., 2018b); bats (Safi and Kerth, 2003); reptiles and amphibians (Mason and Parker, 2010; Saporito et al., 2012); and birds (Leclaire et al., 2012; Whittaker et al., 2013). These advances have particularly been supported in humans for evolutionary and medical purposes (e.g. Havlicek and Roberts, 2009; Penn and Potts, 1998; Roberts et al., 2011; Vaglio, 2009; Winternitz and Abbate, 2015). These recent findings have opened a new era of research on primate chemosignalling, at behavioural, chemical, and genetic levels.

I.3.2. Current research on primate chemosignalling

Callitrichids, i.e. marmosets and tamarins, kept in laboratory conditions have provided a good model for behavioural, physiological, and chemical studies on primate chemosignalling (**Fig. I.7**), as evidenced by the important work of Smith and Epple starting in the 1970s (**Table I.2**). More recently, other captive primate populations allowed for in-depth chemosignalling studies, such as the research on strepsirrhines, i.e. the lemurs and lorises, led by Drea and co-workers at the Duke Lemur Center in North Carolina, USA (**Table I.2**, highlighted in orange). Although scent-marking behaviour had been described in the wild in several primate clades (e.g. in strepsirrhines, Jolly, 1966; and callitrichids, Heymann, 1998; Lazaro-Perea, Snowdon and Arruda, 1999), only after the turn of the century did it become a quite strong focus of research (Heymann, 2006b). One long-term study of sympatric tamarins in northern Peru led by

Heymann from the German Primate Centre, and co-workers (**Table I.2**, highlighted in green), together with a number of short-term studies on lemurs, and a handful of studies of semi-free range catarrhines, i.e. Old World primates, from Setchell and co-workers, constitute the core of existing published knowledge of scent-marking in wild primates (**Table I.2**). Captive studies continue to be the main conditions for studies on chemosignalling in primates (**Fig. I.7**), facilitating: **1.** behavioural observations, as scent-marking behaviour is easier to record than in natural conditions; **2.** behavioural bioassays, as experimental work is much more easily controlled in a captive environment; and **3.** chemical analyses of scent-gland secretions and/or deposited scent-marks, as sample collection and storage is more convenient in captivity (Drea et al. 2013). Today, conservation breeding programmes of rare primate species are providing an extra incentive for deciphering their chemical communication both in the wild and in captivity (Dehnhard, 2011; Jennings and Prescott, 2009; Swaisgood and Schulte, 2010).

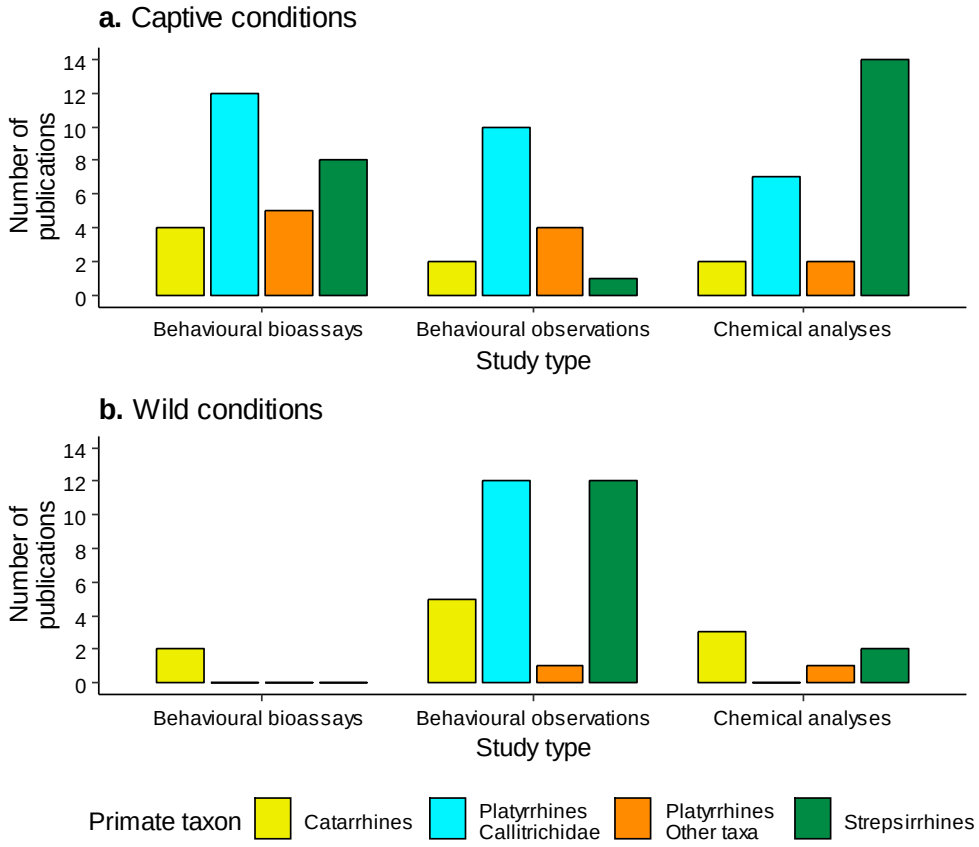


Figure I.7. Number of publications between 1970–2018 in the field of non-human primate chemosignalling, by study type and primate taxon, in **a.** captive conditions, and **b.** wild conditions. Reviews are not included.

Table I.2. Summary of published work between 1970–2018 in the field of non-human primate chemosignalling, by primate taxon, study conditions (i.e. captive or wild conditions) and study type (i.e. behavioural observations, behavioural bioassays, or chemical analyses). Key studies on lemurs carried out at the Duke Lemur Center, and on wild tamarins carried out by Heymann and co-workers in northern Peru, are highlighted in orange and green, respectively. Reviews are not included.

Primate taxa and families	Study condition	Study type	# Publi.	Main species studied and references
<u>Strepsirrhini</u> Lemuridae, Indriidae, Daubentoniidae & <u>Tarsiiformes</u> Tarsiidae (1/2)	Captive	Behavioural observations	1	Ring-tailed lemur, *Lemur catta* (Kappeler, 1998)
		Behavioural bioassays	8	Black-and-white ruffed lemur, *Varecia variegata* (Rushmore, Leonhardt and Drea, 2012) Coquerel's sifaka, *Propithecus coquereli* (Rushmore, Leonhardt and Drea, 2012) Gray mouse lemur, *Microcebus murinus* (Aujard and Némoz-Bertholet, 2004) Ring-tailed lemur (Charpentier et al., 2010; Greene et al., 2016a; Kulahci et al., 2014; Mertl-Millhollen, 2006; Rushmore, Leonhardt and Drea, 2012; Scordato and Drea, 2007) Pygmy slow loris, *Nycticebus pygmaeus* (Fisher, Swaisgood and Fitch-Snyder, 2003a; b)
		Chemical analyses	14	Aye-aye, *Daubentonia madagascariensis* (DelBarco-Trillo et al., 2013) Brown lemurs, *Eulemur* spp. (DelBarco-Trillo et al., 2012) Coquerel's sifaka (Greene & Drea 2014; Hayes et al. 2004) Ring-tailed lemur (Boulet, Charpentier and Drea, 2009; Boulet et al., 2010; Charpentier, Boulet and Drea, 2008; Charpentier et al., 2010; Crawford and Drea, 2015; Crawford, Boulet and Drea, 2011; Knapp, Robson and Waterhouse, 2006; Scordato, Dubay and Drea, 2007) 12 Strepsirrhine spp. (Delbarco-Trillo et al., 2011; 2014)

Table I.2. Continued (1/4).

Primate taxa and families	Study condition	Study type	# Publi.	Main species studied and references
<u>Strepsirrhini</u> Lemuridae, Indriidae, Daubentoniidae & <u>Tarsiiformes</u> Tarsiidae (2/2)	Wild	Behavioural observations	12	Bamboo lemur, *Hapalemur meridionalis* (Eppley, Ganzhorn and Donati, 2016) Coquerel's sifaka (Lewis 2005; 2006; Lewis & Van Schaik 2007) Crowned sifaka, *P. coronatus* (Ramanamisata et al., 2014) Milne-Edwards' sifaka, *P. edwardsi* (Pochron et al., 2005a; b) Red lemur, *Eulemur rufus* (Gould and Overdorff, 2002) Ring-tailed lemur (Gould and Overdorff, 2002; Mertl-Millhollen, 2006; Palagi and Norscia, 2009; Walker-Bolton and Parga, 2017) Pygmy tarsier, *Tarsius pumilus* (Grow and Gursky-Doyen, 2010)
		Behavioural bioassays	0	NA
		Chemical analyses	2	Milne-Edwards' sifaka (Hayes et al. 2006; Morelli et al. 2013)
<u>Platyrrhini</u> Callitrichidae (1/2)	Captive	Behavioural observations	10	Common marmoset, *Callithrix jacchus* (Epple, 1970, 1972; Massen, Šlipogor and Gallup, 2016) Cotton-top tamarin, *Saguinus oedipus* (French and Cleveland, 1984; French, Abbot and Snowdon, 1984; Heistermann et al., 1989) Pygmy marmoset, *Cebuella pygmaea* (Converse et al., 1995) Red-bellied tamarin, *S. labiatus* (Smith and Gordon, 2002) Saddleback tamarin, *Leontocebus* sp. (Epple, 1981, 1982)

Table I.2. Continued (2/4).

Primate taxa and families	Study condition	Study type	# Publi.	Main species studied and references
Platyrrhini Callitrichidae (2/2)	Captive	Behavioural bioassays	12	Common marmoset (Barrett, Abbott and George, 1990; Kemp and Kaplan, 2012; Smith and Abbott, 1998; Smith et al., 1997; Ziegler et al., 2005, 2012) Cotton-top tamarin (Belcher et al., 1988; Washabaugh and Snowdon, 1998) Red-bellied tamarin (Caine and Weldon, 1989) Saddleback tamarin (Belcher et al., 1986, 1990; Epple, 1981)
		Chemical analyses	7	Common marmoset (Kücklich et al., 2017; Smith et al., 2001b) Cotton-top tamarin (Belcher et al., 1988) Saddleback tamarin (Belcher et al., 1986, 1990; Epple et al., 1981; Yarger et al., 1977)
	Wild	Behavioural observations	12	Black-tufted marmoset, *C. penicillata* (Oliveira and Macedo, 2010) Common marmoset (Lazaro-Perea, Snowdon and Arruda, 1999) Golden lion tamarin, *Leontopithecus rosalia* (Franklin et al., 2007; Miller, Laszlo and Dietz, 2003; Snyder, 1972) Moustached tamarin, *S. mystax* (Heymann 1998; 2000; 2001; Huck et al. 2004) Saddleback tamarin (Bartecki and Heymann, 1990; Heymann, 2001; Lledo-Ferrer et al. 2010; 2011)
		Behavioural bioassays	0	NA
		Chemical analyses	0	NA

Table I.2. Continued (3/4).

Primate taxa and families	Study condition	Study type	# Publi.	Main species studied and references
Platyrrhini **Other taxa**	Captive	Behavioural observations	4	Common woolly monkey, *Lagothrix lagothricha* (White et al., 2000) Southern brown howler monkey, *Alouatta guariba clamitans* (Braga Hirano, Coelho Correa and Goncalves de Oliveira, 2008) Nancy Ma's night monkey, *Aotus nancymaae* (Wolovich and Evans, 2007) White-faced saki monkey, *Pithecia pithecia* (Homburg, 1989)
		Behavioural bioassays	5	Common squirrel monkey, *Saimiri sciureus* (Laska et al., 2007; Laska and Hudson, 1995; Laska, Seibt and Weber, 2000; Laska, Wieser and Hernandez Salazar, 2005) Geoffroy's spider monkey, *Ateles geoffroyi* (Laska et al., 2004; 2007; Laska, Wieser and Hernandez Salazar, 2005)
		Chemical analyses	2	Nancy Ma's night monkey (MacDonald et al., 2008; Spence-Aizenberg et al., 2018)
	Wild	Behavioural observations	1	Common woolly monkey (Di Fiore, Link and Stevenson, 2006)
		Behavioural bioassays	0	NA
		Chemical analyses	1	Azara's owl monkey, *A. azarae* (Spence-Aizenberg et al., 2018)

Table I.2. Continued (4/4).

Primate taxa and families	Study condition	Study type	# Publi.	Main species studied and references
Catarrhini **Cercopithecidae, Hominidae**	Captive	Behavioural observations	2	De Brazza's monkey, *Cercopithecus neglectus* (Zschoke and Thomsen, 2014) Diana monkey, *C. diana* (Zschoke and Thomsen, 2014) Hamlyn's monkey, *C. hamlyni* (Zschoke and Thomsen, 2014) Siamang, *Symphalangus syndactylus* (Geissmann, 1987)
		Behavioural bioassays	5	Chimpanzee, *Pan troglodytes* (Henkel and Setchell, 2018) Japanese macaque, M*acaca fuscata* (Rigaill et al., 2017) Southern pig-tailed macaque, *M. nemestrina* (Laska et al., 2004, 2007; Laska, Wieser and Hernandez Salazar, 2005)
		Chemical analyses	2	Chimpanzee (Matsumoto-Oda et al., 2003) Mandrill, *Mandrillus sphinx* (Vaglio et al., 2016)
	Wild / Semi-free range	Behavioural observations	5	Mandrill (Charpentier et al., 2013) Olive baboon, *Papio anubis* (Rigaill et al., 2013) Vervet monkey, *Chlorocebus aethiops* (Freeman et al., 2012) Western lowland gorilla, *Gorilla gorilla* (Klailova and Lee, 2014; Masi and Bouret, 2015)
		Behavioural bioassays	2	Rhesus macaque, *M. mulatta* (Henkel et al., 2015; Weiß et al., 2018a)
		Chemical analyses	3	Mandrill (Setchell et al. 2010; 2011) Rhesus macaque (Birkemeyer et al., 2016)

I.4. Study outline

I.4.1. Presentation of the study

The present study combines behavioural and semiochemical data on both captive and wild callitrichids (family Callitrichidae, New World primates), in an attempt to decipher some of the environmental, social, and individual aspects of their chemical communication. Moreover, the study is aimed at developing techniques for the analysis of primate semiochemicals, in an effort to contribute to innovative knowledge in the field.

The Callitrichidae family is a monophyletic group of New World primates, composed of the tamarins (*Saguinus* and *Leontocebus* spp.), the lion tamarins (*Leontopithecus* spp.), the marmosets (*Mico* and *Callithrix* spp.), the pygmy marmosets (*Cebuella* spp.), and the Goeldi's monkey, *Callimico goeldii* (Rylands and Mittermeier, 2013; Rylands et al., 2016). Callitrichids are small, long-tailed primates, characterized by claw-like nails on all digits except the hallux, and a tendency to twin (except for *Callimico*). They range from Panama to southern Brazil, and are found in a variety of habitat types, from tall primary forests to farmlands (Sussman, 2003). Callitrichids constitute a good model for the study of olfactory communication, as they are known to rely a lot on odour signals, notably thanks to a well-developed vomeronasal organ, which is known to be at least partially functional (Evans, 2006; Smith et al., 2011). In callitrichids, olfactory signals are produced via three specialized scent-glands, and conspicuously deposited on branches and lianas in the environment (scent-marking) or on the body of a conspecific (allomarking). Conspecifics investigate each other's scent-marks by sniffing, muzzle-rubbing, licking, or overmarking scented spots (Smith et al., 1997). Several functions have been attributed to callitrichid scent-marking behaviour, including **1.** advertisement of identity, and of reproductive and dominance status; **2.** territorial advertisement and defence; and **3.** spatial orientation and signalling of food resource location (Epple, 1986; Lazaro-Perea, Snowdon and Arruda, 1999; Miller, Laszlo and Dietz, 2003; reviewed in Heymann, 2006a). These functions will be further detailed in the next section.

The captive part of the study took place between March 2017–March 2018 in three British zoos, members of the British and Irish Association of Zoos and Aquariums (BIAZA). I recorded scent-marking

behaviour of several groups of emperor tamarins, *Saguinus imperator subgrisescens* (further referred to as *S. imperator*), cotton-top tamarins, *S. oedipus*, and silvery marmosets, *Mico argentatus*. The results of this behavioural study constitute **Chapter 2** of the present thesis.

During these observations, I collected scent samples from voided urine and naturally deposited scent-marks inside the enclosures. Samples from some of the animals' scent-gland secretions were additionally collected when undergoing routine husbandry procedures. I analysed the scent samples at Anglia Ruskin University (ARU) by SPME–GC-MS. In addition, I used a subset of these samples to experimentally test the temporal stability of sample chemical composition. This captive semiochemical study is presented in **Chapter 3**.

The study of wild callitrichids was conducted in June 2017 at Estación Biológica Los Amigos (EBLA) in south-eastern Peru, on two sympatric species of tamarin, emperor tamarins and Weddell's saddleback tamarins, *Leontocebus weddelli*. Scent samples were collected from the tamarins' scent-glands and skin during an annual capture-and-release programme at this site, which I analysed *in situ* using a new generation portable GC-MS device. Additional emperor tamarin samples were collected in June 2018 and transported to ARU, where I analysed them in the laboratory, in order to compare the two analytical methods. Results from this study are described in **Chapter 4**.

This study provides an important approach in directly comparing **1.** scent-marking behaviour and semiochemistry in several species of callitrichids; **2.** the chemical composition of scent-gland samples from captive and wild tamarins; and **3.** the use of laboratory-based and *in situ* analytical methods for the analysis of callitrichid scents. Besides providing valuable insight on the chemical composition of the signals conveyed via scent communication, I hope the present study can offer a guideline for the captive breeding and husbandry of rare callitrichids and other mammals. These ideas, following a discussion of the results found in both captive and wild conditions, are discussed in **Chapter 5**.

I.4.2. Research questions

The research questions considered in this study can be divided along three axes: **1.** questions relating to callitrichid scent-marking behaviour; **2.** questions relating to differences in the chemical composition of callitrichid scent samples; and **3.** questions relating to the semiochemistry techniques used for the analyses of callitrichid scents. These research questions are further summarized in **Table I.3**.

I.4.2.1. Questions relating to scent-marking behaviour

Q1. Are there differences in scent-marking behaviour at the levels of species, group, sex, reproductive status, and/or the individual?

In wild callitrichids, overlaps between different groups' home ranges are frequent, sometimes even between two or three sympatric species (Smith, 1997; Watsa, 2013). Hence, we might expect differences in scent-marking activity between species, and between groups, reflecting different strategies of communication to ensure that messages are transmitted to the correct receivers. This was found by Heymann (2001) in sympatric moustached tamarins, *Saguinus mystax*, and Geoffroy's saddleback tamarins, *Leontocebus nigrifrons* (formerly *S. fuscicollis*, recently reassigned, Rylands et al., 2016). Differences in scent-marking behaviour at the level of species may also indicate variable relative importance of olfactory communication compared with other communication modalities (Higham and Hebets, 2013). I will explore the differences in scent-marking frequency between species and groups of the captive callitrichids studied.

Dominance and reproductive status are highly entwined in callitrichids, owing to their cooperative breeding system (Huck et al., 2005). As olfactory cues are involved in reproduction (Huck, Löttker and Heymann, 2004; Ziegler, 2013), we can predict reproductively active individuals to scent-mark more often than the non-reproductive and immature ones, as was found in captive common marmosets (Epple 1972). Scent-marking may notably play a role in the reproductive suppression of callitrichid subordinate females, occurring through both behaviour and chemical cues from the dominant female (e.g. in common marmosets, Abbott et al., 1998; Barrett, Abbott and George, 1990; Saltzman et al., 1997; Ziegler and Sousa, 2002; Ziegler, 2013; golden lion tamarins, *Leontopithecus rosalia*,

French and Stribley, 1985; cotton-top tamarins, Heistermann et al., 1989; Savage, Ziegler and Snowdon, 1988; and pygmy marmosets, *Cebuella pygmaea*, Spurlock, 2001; reviewed in Beehner and Lu, 2013). The existence of strict reproductive suppression in other callitrichid species is still unclear, although probable (Watsa, 2013). However in studies on wild common marmosets (Lazaro-Perea, Snowdon and Arruda, 1999; Sousa et al., 2005), and moustached tamarins (Huck et al., 2005), subordinate females scent-marked more frequently than the reproductive female, indicating a possible function of scent-marking in mate attraction and intrasexual competition (reviewed in Heymann, 2006a). I will test the influence of sex and reproductive conditions on the frequency of scent-marking behaviour in captive callitrichids and inspect individual differences.

Q2. What is the social context of scent-marking behaviour in captive callitrichids?

Scent-marking is very common in callitrichids, reported both in the wild and in captivity (**Table I.2**; Epple, 1974a; Heymann, 2006a). As for many other behaviours in social species, scent-marking, which is a conspicuous behaviour, may represent a visual signal in itself, in addition to the olfactory message produced (Johnstone, 1996; Liebal et al., 2014). If this is the case, we can expect scent-marking behaviour to be performed more frequently in the presence of conspecifics and located on substrates particularly visible to the rest of the congeners present. We can also predict callitrichid scent-marking activity to be influenced by the identity of conspecifics present, since effort in marking behaviour may be directly linked to the potential receivers, or to other signalling individuals. This would indicate a directed transmission of individual signals between two given animals via olfactory communication. I will test these hypotheses in captive callitrichids.

Q3. How much variation is found in captive callitrichid scent-mark deposition?

Scent-marking in mammals can differ considerably in its mode of deposition, duration, and the type of mark. Notably, different fluids can sometimes be mixed together, which is likely to modify the signal content of the deposited mark, such as in the case of female giant pandas known to use a mixture of urine, vaginal discharge, and anogenital scent-gland

secretions as scent-marks (Hagey and Macdonald, 2003). Moreover, most mammals possess scent-glands on diverse regions of their body. Callitrichids have three distinct specialized scent-glands on the anogenital, suprapubic, and sternal regions of their body. Scent-glands may be used in different contexts to convey different signals (Heymann, 2001). Furthermore, variation in scent-marking duration may reflect differences in the amount of secretions deposited during a scent-marking event, which could result in the transmission of distinct signals. Such potential disparities in scent-marking activity illustrate the variety and complexity of the chemical cues mediating social communication. I will assess the diversity of scent-marking characteristics in captive callitrichids by comparing types and duration of scent-marking, as well as scent-gland use.

Q4. What are the temporal and spatial patterns of scent-marking behaviour in captive callitrichids?

Scent-marking in callitrichids may be associated with territorial defence and food resource marking (Miller, Laszlo and Dietz, 2003; Mitani and Watts, 2005). Therefore, we can expect this behaviour to occur more frequently in the wild than in captivity given the fact that ***i.*** home ranges are obviously much larger; ***ii.*** food resources are unevenly distributed and far away from each other; and ***iii.*** physical encounters with potential rival groups are possible. In a captive environment, we can anticipate scent-marking behaviour to be performed more frequently in association with feeding activities, which would be consistent with a role in the signalling of food resources. I will describe the spatiotemporal patterns of scent-marking behaviour in captive callitrichids.

I.4.2.2. Questions relating to differences in the chemical composition of callitrichid scents

Q5. To what extent does naturally deposited scent-mark chemical composition differ from that of scent-gland secretions, and from urine?

The chemical components of scent-gland secretions after scent-marking potentially mix with other body fluids such as urine, and with chemicals from conspecifics, plants and microorganisms present in the environment (Archie and Theis, 2011; Ezenwa and Williams, 2014).

Moreover, volatile chemicals naturally change and re-associate as soon as they are liberated in the air (Greene et al., 2016a; Charpentier et al., 2012). We can therefore expect the chemical composition of scent-mark samples to be different from those collected directly from the scent-gland of the animal. I will test this assumption in captive callitrichids. Incidentally, a change over time of the chemical composition of a scent-mark can be biologically meaningful, potentially providing the receiver with information on how old the mark is, as well as the context of its deposition (Ezenwa and Williams, 2014; Müller-Schwarze, 2006). Urine on the other hand, may convey olfactory cues as a by-product of its primary role of excretory body fluid, which is well known in many mammals such as dogs, *Canis lupus domesticus*, and rats, *Rattus* spp. (Wyatt, 2014a). Scent-gland secretions, in turn, are purposely deposited in the environment as a discrete chemical signal. Urine could thus represent the ancestral state of chemosignalling, and glandular secretions a more novel chemosignalling mode shown only by some animal taxa (Apps, Mmualefe and Weldon McNutt, 2012; Hagey and Macdonald, 2003). Hence, we can anticipate callitrichid scent-gland secretion chemical composition to be ***i.*** different, and ***ii.*** more complex, than that of urine, owing to its specific chemosignalling function. Alternatively, urine might be chemically more complex than scent-gland secretions, since it is an aqueous mixture of many residual chemicals from diverse reaction chains. Lastly, urine and scent-gland secretions might present similar volatile compounds, but in different quantities. Urine and scent-marks are both deposited in the environment and subject to decay and mixing. The balance of volatile and semi- or non-volatile compounds serving as olfactory cues may change over time, as the most volatile compounds rapidly disperse into the air (Müller-Schwarze, 2006). Unfortunately non-volatile compounds cannot be detected by the analytical technique used in the present study (i.e. headspace GC-MS), therefore only a partial picture of the chemical signals can be obtained. I will compare the chemical composition of captive callitrichid deposited scent-marks, scent-gland secretions, and urine, analysed by SPME–GC-MS.

Q6. Do callitrichid scent-gland secretions, scent-marks, and urine contain chemical signatures at the levels of species, group, sex, reproductive status, and/or the individual?

As mentioned in **Q1**, chemosignals may be used to advertise territoriality and identity, as well as reproductive and dominance status. In primates in particular, it has been shown that chemical signatures are found in the chemical profiles of deposited scents at the levels of species (e.g. in urine of brown lemurs, *Eulemur* spp., DelBarco-Trillo and Drea, 2014; in glandular secretions of ring-tailed lemurs and Coquerel's sifakas, *Propithecus coquereli*, Hayes, Morelli and Wright, 2004), and group (e.g. in deposited scent-marks of mandrills, *Mandrillus sphinx*, Vaglio et al., 2016), which might reflect different territorial strategies, and ensure interspecific recognition in sympatric species. Moreover, chemical signatures at the levels of sex (e.g. in glandular secretions of owl monkeys, *Aotus* spp., Spence-Aizenberg et al., 2018), reproductive status (e.g. in glandular secretions of Coquerel's sifakas, Greene and Drea, 2014), and the individual (e.g. in glandular secretions of common marmosets, Smith et al., 2001b), may indicate a role of olfactory communication in mate choice, dominance, and reproductive suppression in callitrichids. I will examine these assumptions in both captive and wild callitrichids.

Q7. Are there differences in chemical composition between samples from different scent-glands in wild tamarins?

Tamarins possess three types of scent-glands, anogenital, suprapubic, and sternal, of comparable histology (Epple et al., 1993; Fontani et al., 2014; Moraes et al., 2006; Perkins, 1966). Previous work on the ring-tailed lemur has identified differences in the chemical composition of genital and brachial scent-gland secretions, suggesting that different messages may be conveyed by each of these glands (Greene et al., 2016a; Scordato, Dubay and Drea, 2007). I will compare the chemical composition of the different glands of individuals of two wild sympatric tamarin species.

Q8. Are there differences in chemical composition between scent-gland samples from captive and wild emperor tamarins?

Chemosignals used in animal olfactory communication can either be by-products of essential biochemical pathways, derived from the

environment, or produced by commensal bacteria (Archie and Theis, 2011; Ezenwa and Williams, 2014). In a captive environment, variations in diet, elements of the enclosure, and husbandry procedures, may influence the commensal bacteria communities of the animals, thus resulting in a range of different compounds being produced. For instance, diet has been shown to have an effect on the production of cuticular hydrocarbons by fruit flies, *Drosophila melanogaster* (Fedina et al., 2012); on the sex pheromones released by male cockroaches, *Nauphoeta cinerea* (South et al., 2011); and on the chemical composition of genital secretions of various strepsirrhine species (Drea et al., 2013); although Baeckens et al. (2017) did not find differences in the chemical composition of femoral gland secretions from lacertid lizards (Lacertidae) subjected to different diets. Another study revealed that captivity altered the diversity of skin lipids produced by red-sided garter snakes, *Thamnophis sirtalis parietalis* (Rudie, 2015). Wild tamarins, which have access to a greater variety of food items, and potentially interact with a greater diversity of organisms (i.e. con- and hetero-specifics, predators, prey, parasites and other microorganisms), may produce ***i.*** different, and ***ii.*** potentially more complex chemical signals, than their captive counterparts. I will compare the chemical composition of samples from wild and captive emperor tamarins.

Q9. Is there a relationship between scent-marking behaviour and semiochemistry in captive callitrichids?

Scent-marking frequency can be assessed from behavioural observations. If differences are found at the levels of species, group, sex, reproductive status, the individual, or between scent-glands for the callitrichid groups considered in this study (see **Q1**), it will be interesting to examine whether the categories of individuals that scent-mark the most also present a more complex, or dissimilar, scent-gland secretion and scent-mark chemical composition (see **Q6**). I will relate chemical and behavioural information on captive callitrichid olfactory communication.

I.4.2.3. Questions relating to technical development for the chemical analysis of callitrichid scents

Q10. What is the pattern of degradation of tamarin scent samples kept at room temperature and subject to repetitive extractions?

Researchers in the field of mammalian semiochemistry usually recommend that scent samples are transported and stored frozen, as they may degrade and change over time, due to the loss of the most volatile compounds, and bacterial activity inside the sample containers (Apps, Weldon and Kramer, 2015; Drea et al., 2013; MacDonald et al., 2008). However, the exact pattern of degradation or change is not yet known for this type of samples. I will investigate this general statement by experimentally testing the decay pattern of tamarin scent-gland secretion samples, subject to repetitive extractions and storage at room temperature.

Q11. How successful was the analytical technique SPME–GC-MS at showing patterns in callitrichid scents; and how could it be improved?

The choice of SPME–GC-MS (**Table I.1**) for the analysis of callitrichid scent samples was motivated by ***i.*** a careful review of existing work using this technique (e.g. Curran et al., 2007; Goodwin et al., 2006; Probert, Jones and Ratcliffe, 2004; Tait et al., 2014; Zagrobelny et al., 2015; see section I.2.2); and ***ii.*** an important process of method optimization on the available instrumentation at ARU throughout the first year of this project. The chromatography results obtained using this technique have both their advantages and limitations, which I will assess and discuss.

Q12. How successful was the use of the Torion® T-9 GC-MS for the analysis of wild tamarin scent-gland secretions; and how could it be improved?

As outlined by Drea et al. (2013), one of the principal difficulties of semiochemical analyses in wild conditions is the prior storage and transportation of samples in cold conditions, to avoid sample degradation (see **Q10**). The Torion® T-9 (PerkinElmer, 2016), a portable GC-MS developed for environmental analyses of air and water in war zones, offers a solution to this problem by allowing *in situ* chemical analyses of freshly collected samples. The Torion®, associated with SPME, was employed to

analyse the chemical composition of scent-gland secretions from wild tamarins. I will assess and discuss the chromatography results obtained using this technique, in comparison with laboratory analyses of similar samples using a conventional bench-top GC-MS.

Table I.3. Summary of the three axes of research developed in the present doctoral project, their associated research questions, and corresponding thesis chapters.

Research axis	Q #	Research question	Thesis chapter
1. Captive callitrichid scent-marking behaviour	**Q1.**	Are there differences in scent-marking frequency at species, group, sex, reproductive status, and individual levels?	Chap. 2
	Q2.	What is the social context of scent-marking behaviour?	Chap. 2
	Q3.	How much variation is found in scent-marking deposition?	Chap. 2
	Q4.	What are the temporal and spatial patterns of scent-marking behaviour?	Chap. 2
2. Captive and wild callitrichid semiochemistry	**Q5.**	Does scent-mark chemical composition differ between scent-gland secretions, scent-marks, and urine, in captivity?	Chap. 3
	Q6.	Do callitrichid scents contain chemical signatures at the levels of species, group, sex, reproductive status, and/or the individual?	Chap. 3-4
	Q7.	Are there differences in chemical composition between different scent-glands in wild tamarins?	Chap. 4
	Q8.	Are there differences in chemical composition between scent-gland samples from captive and wild emperor tamarins?	Chap. 4-5
	Q9.	Is there a relationship between scent-marking behaviour and semiochemistry in captivity?	Chap. 5
3. Semiochemistry techniques	**Q10.**	What is the pattern of degradation of scent samples?	Chap. 3
	Q11.	How successful was the use of SPME–GC-MS in the captive study?	Chap. 4
	Q12.	How successful was the use of Torion® T-9 portable GC-MS in the wild study?	Chap. 4

Chapter II – Scent-marking behaviour in captive callitrichids

Abstract

This chapter describes and compares scent-marking activity recorded *ad libitum* in five captive groups of callitrichids. First, I observed patterns of identity in scent-marking activity at the levels of species, group, sex, reproductive status, and the individual. I found differences between species and groups, possibly partially explained by differences between captive environments, as well as group size and composition, at the various study sites. Female tamarins scent-marked more than males, suggesting a role of scent-marking in advertising female reproductive status, otherwise concealed in callitrichids. Reproductive females tended to produce the most scent-marks, indicating a possible role of chemosignalling in intrasexual competition and reproductive suppression in these cooperative breeding primates. Furthermore, the influence of the identity of conspecifics present at the time of scent-marking reflected potentially distinct strategies adopted by individuals when exchanging chemical signals. Second, I found differences in scent-marking type and duration, and scent-gland use, between groups. Tamarins mainly marked using their anogenital scent-gland; marmosets using their suprapubic gland. Both callitrichid taxa sometimes combined urine with their scent-marks, and marmosets often deposited rapid scent-marks after tree-gouging, thus potentially modifying the chemosignals conveyed. Lastly, I observed a variation in the spatiotemporal pattern of scent-marking behaviour. Time of day had little influence on scent-marking activity, although scent-marking tended to be less frequent in the morning. Horizontal and inclined substrates in indoor enclosure areas were favoured for depositing scent-marks. These results support the view of a main function of scent-marking behaviour in callitrichids in advertisement of identity, as well as of reproductive and dominance status. Moreover, the different scent-marking characteristics recorded in this study emphasize the diversity and complexity of chemosignals transmitted via scent-marking.

II.1. Introduction and hypotheses

II.1.1. Scent-marking behaviour in the Callitrichidae: mechanisms and functions

Scent-marking is very common in callitrichids, reported both in captivity (Epple, 1972; 1973; 1974a) and in the wild (Heymann, 2006a; b). Callitrichids produce scent signals by depositing glandular secretions on selected branches in their environment, produced by specialized scent-glands on the anogenital, suprapubic, and sternal area of their body. Sometimes, the secretions are mixed with urine, faeces or genital secretions, and produce a potentially distinct scent signal (Sutcliffe and Poole, 1978; in Smith et al., 2001b). Scent-marks provide durable olfactory cues that potentially remain long after departure of the signaller, as opposed to immediate visual or acoustic signals (Alcock, 2013; Liebal et al., 2014). However, scent signals may change as soon as they are deposited, owing to the loss of volatile compounds and to bacterial activity (Archie and Theis, 2011; Theis et al., 2013; see **Chapter 3**). Moreover, scent-marking is a particularly noticeable behaviour in callitrichids, in which the animals ostensibly crouch down and repeatedly rub their scent-gland area against the substrate (usually a branch), often in the presence of conspecifics (Lazaro-Perea, 2001; pers. obs.). This conspicuousness of scent-marking behaviour may serve to attract the attention of one or several particular receivers. It may also constitute a visual signal in itself, regardless of the chemical composition of the secretions and/or urine deposited (Johnstone, 1996). Conspecifics may investigate each other's scent-marks by sniffing, muzzle-rubbing, licking, or over-marking scented spots (Smith et al., 1997). Some aspects of scent-marking behaviour include the identity of the signaller, scent-mark characteristics (e.g. scent-gland used, presence of urine mixed with secretions, and duration of the marking), the choice of substrate to mark, the number and identity of potential receivers, and the investigatory response elicited.

Several functions have been attributed to animal scent-marking behaviour, including: **1.** advertisement of identity at the levels of species, group, sex, age, and/or the individual, as well as of reproductive and dominance status; **2.** territorial advertisement and defence; and **3.** spatial orientation and signalling of food resource location (Müller-Schwarze, 2006; Thiessen and Rice, 1976; Wyatt, 2014a). These functions also apply to the

social and sexual lives of primates, as reviewed by Dominy, Ross and Smith (2004), Heymann (2006a), and Snowdon et al. (2006). In the Callitrichidae (i.e. the marmosets and tamarins), the first function of scent-marking behaviour, i.e. advertisement of identity, reproductive and dominance status, may serve a role in the regulation of social and reproductive dominance in this cooperative breeding taxon, such as in intrasexual competition and mate choice (Abbott et al., 1998; Savage, Ziegler and Snowdon, 1988; Smith et al., 2001b). The second function, i.e. territorial advertisement and defence, is the most commonly referred to in mammalian studies (Albone and Shirley, 1984). However, there is currently a debate about the role of scent-marking in territoriality in the Callitrichidae, as patterns of scent-marking do not match home range boundaries in sympatric Geoffroy's saddleback tamarins, *Leontocebus nigrifrons* (formerly *Saguinus fuscicollis*, recently reassigned, Rylands et al., 2016), and moustached tamarins, *Saguinus mystax* (Lledo-Ferrer, Peláez and Heymann, 2011, 2012; Roberts, 2012). Finally, the third function, i.e. spatial orientation and signalling of food resource location, has been established in wild groups of common marmosets, *Callithrix jacchus* (Lazaro-Perea, Snowdon and Arruda, 1999; Thompson et al., 2018), and golden lion tamarins, *Leontopithecus rosalia* (Miller, Laszlo and Dietz, 2003). However, this function is nearly impossible to investigate in captive conditions, where space and food resources are so constrained.

II.1.2. Aims and hypotheses

This chapter investigates variation in scent-marking activity in five groups of captive callitrichids, of three different species, housed at three different zoos. First, I compared the frequency of scent-marking behaviour between the different species and groups studied, as well as at the levels of sex, reproductive status, and the individual. Since the home ranges of multiple callitrichid groups, and sometimes even of two or three sympatric species, frequently overlap (Heymann, 2006a), we can predict differences in scent-marking activity between species, and between groups, reflecting different strategies of communication to ensure that signals are conveyed to the correct receivers. Differences in scent-marking behaviour at the level of species may also indicate a variable relative importance of chemosignalling compared with other communication modalities (Higham

and Hebets, 2013; Partan, 2013). Moreover, as scent-marking behaviour in callitrichids has been shown to play a role in the advertisement of reproductive and dominance status (Heymann, 2006a), differences in scent-marking activity are expected at the level of sex, reproductive status, and the individual, as introduced in **Chapter 1**, section I.4.2. Notably, dominant, reproductive females may scent-mark more as a means to indicate their reproductive status to potential mates (Epple, 1972), and to suppress ovulation in the subordinate females of the group, as scent-marking has been shown to play a role in the reproductive suppression of subordinate females in this taxon (Beehner and Lu, 2013). I also inspected the 'social context' of scent-marking activity, defined as the influence of the number and identity of conspecifics surrounding the signaller while scent-marking, as well as the investigatory response elicited by scent-marks. We can hypothesize that most scent-marking may happen in the presence of conspecifics, where the conspicuous scent-mark deposition would easily attract the attention of particular receivers, and that marking from a given signaller may be directed to a particular conspecific (Kappeler, 1998).

Second, I investigated variation in scent-mark deposition, within and across callitrichid groups. In particular, I compared the use of glandular secretions only, with the use of a mixture of glandular secretions and urine. I also explored differences in the use of scent-gland (i.e. anogenital, suprapubic or sternal), and in the duration of scent-mark deposition. All three characteristics may influence the range and concentration of chemical components deposited when scent-marking, thus potentially conveying different scent signals. The variation in callitrichid chemosignalling will be further investigated at a chemical level in **Chapters 3**–**4**.

Finally, I examined the temporal and spatial variation of scent-marking activity. As is the case in other mammals such as canids and felids, which tend to deposit urine marks on prominent rocks and trunks (Apps, Weldon and Kramer, 2015; Soso and Koziel, 2017), we can expect callitrichids to preferentially scent-mark on highly accessible substrates, commonly visited by conspecifics, thus more easily detectable by potential receivers. Callitrichids might also place their scent-marks in key locations for the signalling of food resources or sleeping sites, and in association with feeding or social activities at certain times of day. However this hypothesis is difficult to test in captivity, where space is so constrained.

II.2. Methods

II.2.1. Study sites and species

Scent-marking behaviour was studied in captive bearded emperor tamarins, *Saguinus imperator subgrisescens* (referred to as *Saguinus imperator* in the rest of this study; Deville, 1849; Rylands et al., 2016), cotton-top tamarins, *S. oedipus* (Linnaeus 1758), and silvery marmosets, *Mico argentatus* (formerly *Callithrix argentata*, Linnaeus 1758; Rylands, 1993). A group of six emperor tamarins and a group of eight silvery marmosets housed at Twycross Zoo (TZ) were observed in April and October 2017, respectively. A group of six cotton-top tamarins housed at Paradise Wildlife Park (PWP) was studied in September 2017. Finally, a group of four cotton-top tamarins and a pair of emperor tamarins housed at Drayton Manor Park (DMP) were observed in January and February 2018, respectively. The three study sites are all members of the British and Irish Association of Zoos and Aquariums (BIAZA). This project received approval from the Faculty of Science and Engineering Departmental Research Ethics Panel committee at Anglia Ruskin University (DREP), BIAZA, and each of the individual study sites.

Individuals in each callitrichid group were classified as reproductive adults (i.e. fully sexually mature individuals), subordinate adults (i.e. offspring of the reproductive pair, of over 18 months old, probably sexually mature but not having reproduced), juveniles (i.e. offspring of the reproductive pair, of less than a year old), and an infant (i.e. offspring of the reproductive pair, not yet weaned; **Table II.1**).

Table II.1. Composition of the five callitrichid groups included in the study. Photographs show adults of the three species.

Species common & scientific name	Study site	Group size	Group composition	Age (years + months)
Emperor tamarin, *Saguinus imperator* 	Drayton Manor Park	2	Reproductive ♀	3Y 5M
			Reproductive ♂	4Y 8M
	Twycross Zoo	6	Reproductive ♀	5Y 7M
			Reproductive ♂	7Y 11M
			Subordinate ♀	1Y 9M
			Subordinate ♂	1Y 9M
			Juvenile ♀	0Y 6M
			Juvenile ♂	0Y 6M
Cotton-top tamarin, *Saguinus oedipus* 	Drayton Manor Park	4	Reproductive ♀	4Y 8M
			Reproductive ♂	4Y 3M
			Juvenile ♀	0Y 10M
			Juvenile ♂	0Y 10M
	Paradise Wildlife Park	6	Reproductive ♀	11Y 4M
			Reproductive ♂	10Y 7M
			Subordinate ♀	2Y 7M
			Subordinate ♂	1Y 11M
			Juvenile ♀	0Y 5M
			Juvenile ♂	0Y 5M
Silvery marmoset, *Mico argentatus* 	Twycross Zoo	8	Reproductive ♀	7Y 7M
			Reproductive ♂	11Y 0M
			Older subordinate ♀	2Y 11M
			Subordinate ♀	2Y 2M
			Subordinate ♂	2Y 2M
			Juvenile ♀	0Y 8M
			Juvenile ♂	0Y 8M
			Infant (sex unk.)	0Y 2M

II.2.2. Recording of scent-marking behaviour and individual proximity measures

II.2.2.1. Scent-marking behaviour

Each callitrichid group was observed for 50 hours over a ten day period, except for the group of silvery marmosets at TZ, which was observed for 45 hours over nine days. One animal was removed from the silvery marmoset group by the veterinarian team on the tenth day of observation, which had an impact on the general behaviour of the group in the following days (pers. obs.), leading to the decision to discard data collected after this event. A single observer collected all observational data, thus limiting the variability of the recordings (Martin and Bateson, 2007). Prior to data collection, at least one day of observation was spent habituating the primates to the observer's continuous presence, and for the observer to learn to visually differentiate individuals within a group. Daily observation time was five hours, divided into five one-hour bouts at random intervals between 09:00 and 16:40. Scent-marking behaviour was clearly noticeable to the human observer, and the location of each of the three scent-glands on the animals' body generally allowed the distinction between anogenital marking (also called circumgenital marking, e.g. Smith et al., 2001b; **Fig. II.1a**), suprapubic marking (**Fig. II.1b**), and sternal marking (**Fig. II.1c**).

Scent-marking events were recorded *ad libitum* for all individuals during each bout of observation. Two occurrences of scent-marking at short time interval were considered as separate events, unless the second occurrence happened within 2 min and on the exact same spot of the first event, in which case it was classified as an overmark in response to the first scent-mark event. The following information was recorded for each scent-marking event: day, time of day, identity of the marker (i.e. species, study site, group, sex, reproductive status, and individual ID), scent-marking type, scent-gland used, duration of the marking, enclosure area and substrate used, presence of conspecifics and identity of the nearest neighbour when present in the same enclosure section at the time of the scent-marking event, and investigatory response to the mark. These categories are described below, and further summarized in **Table II.2**.

Figure II.1. Photographs of scent-marking behaviour in tamarins: **a.** adult female emperor tamarin, *Saguinus imperator*, anogenital scent-marking (right), while being observed by a subordinate male (left); **b.** adult male Weddell's saddleback tamarin, *Leontocebus weddelli*, suprapubic scent-marking (photo credit: Field Projects International); and **c.** adult male emperor tamarin sternal scent-marking.

For statistical purposes, the recorded scent-marking time was further categorized into a factor ‘time of day’, comprising morning (i.e. 09:00–11:30), midday (i.e. 11:31–14:00), and afternoon (i.e. 14:01–16:40). Scent-marking types were defined as either glandular secretions only (i.e. secretions) or secretions mixed with urine (i.e. urine+secretions), where scent-marking was performed just after urination. In addition, silvery marmosets at TZ often performed scent-marking in association with tree-gouging, a natural foraging behaviour in this exudate-feeding species (Rosenberger, 1978; Rylands, 1984, 1985, 1993). For this species, when scent-marking was performed just after gouging and on the very same spot, it was noted as such (i.e. gouging+secretions). Scent-marking duration was recorded as a categorical factor, comprising short (i.e. 1–2 sec), medium (3–6 sec), and long (>6 sec) depositions. The number of enclosure sections and substrate types available varied between zoos, and between enclosures. Therefore, for the purpose of this study the factor enclosure area only included indoor and outdoor areas, and the factor substrate comprised horizontal, inclined, and vertical substrates. The animals were free to access all areas of their enclosure at all times during data collection. Presence of conspecifics was defined as the number of individuals present in the same enclosure section at the time of scent-marking deposition, and therefore potentially having the signalling individual in sight. Importantly here, unlike the factor enclosure area, enclosure section corresponded to the different spaces of both indoor and outdoor areas, divided by walls, wooden panels, or mesh covered with branches, platforms and/or vegetation, preventing visual contact between the signalling animal and its potential receivers. The categories used were one neighbour, >1 neighbour, and none (i.e. no conspecific present in the same enclosure section). Finally, investigatory response by conspecifics, which corresponded to either sniffing, muzzle-rubbing, or overmarking a deposited scent-mark, was recorded as yes (i.e. occurrence of response) or no (i.e. absence of response). Owing to the limited space available to the animals in captivity, investigatory responses were only recorded if they occurred within 2 min of the scent-marking event, and if the receiver’s head approached within 2 cm of the scent-marked spot, in order to avoid recording behaviours having happened only by chance.

Table II.2. Summary of the information recorded for each scent-marking event included in this study.

Information type	Factor recorded	Categories included
Temporal information	Day	Day 1–Day 10 (only 9 days for silvery marmoset group)
	Time of day	Morning (09:00-11:30); midday (11:30-14:00); afternoon (14:00-16:40)
Identity of the signaller	Species	Silvery marmoset; emperor tamarin; cotton-top tamarin
	Study site	DMP; PWP; TZ
	Group	Silvery marmosets at TZ; emperor tamarins at DMP; emperor tamarins at TZ; cotton-top tamarins at DMP; cotton-top tamarins at PWP
	Sex	Male; female
	Reproductive status	Reproductive; non-reproductive
	Individual	See **Table II.1**
Social context	Presence of conspecifics	One neighbour; >1 neighbours; none
	Identity of the nearest neighbour	See **Table II.1**
	Investigatory response	Yes; no
Characteristics of scent-marking event	Scent-marking type	Secretions; urine+secretions; gouging+secretions (only *M. argentatus*)
	Scent-gland used	Anogenital; suprapubic; sternal
	Scent-marking duration	Short; medium; long
Location of scent-marking event	Enclosure area	Indoor; outdoor
	Substrate	Horizontal; inclined; vertical

II.2.2.2. General use of space and proximity to conspecifics

In addition to the *ad libitum* scent-marking behaviour data, the enclosure area used and the identity of the nearest neighbour were recorded for all individuals using group scan sampling every 2 min (Altmann, 1974). This enabled the proportion of time spent in each enclosure area, and in closest presence of each conspecific, to be examined for each individual in the five groups studied.

II.2.3. Statistical analyses

All statistical analyses were performed in R v.3.5.1 operated in RStudio (R Core Team, 2018). Scent-marking frequency was calculated as the number of scent-marking events per sample category (e.g. species, sex, etc.) per one-hour observation bout. First, differences in hourly scent-marking frequency were assessed using non-parametric Kruskal-Wallis rank sum tests of difference (function kruskal.test() in R base package 'stats'), between species, study sites, and groups. Then, Dunn's tests with Bonferroni adjustment (i.e. pairwise tests for multiple comparisons of mean rank sums; function posthoc.kruskal.dunn.test() in R package 'PMCMR'; Pohlert, 2014) were used as post-hoc tests to investigate pairwise differences within the same factors. Only the first nine days of observation were included, to account for the fact that the group of silvery marmosets at TZ was observed for nine days instead of ten.

In order to account for differences between callitrichid groups, Kruskal-Wallis rank sum tests and Dunn's post-hoc tests were then used for each group independently, to perform pairwise comparisons between categories of sex, reproductive status, individual, scent-mark type, scent-gland used, marking duration, presence of conspecifics, investigative response, time of day, enclosure area used, and substrate used. For these group-level comparisons, all 10 days of observations were included in the four tamarin groups studied. Additionally, the choice of enclosure area during scent-marking was related to the general use of space by performing Pearson's χ^2 tests with Yates' continuity correction (chisq.test() in base R package 'stats'). This test compared the mean daily observed scent-marking frequency per callitrichid group in indoor and outdoor enclosure areas, with the expected values given the general use of space. These expected values were calculated as: $SMexp(i) = (B(i) \times \sum SMobs) / \sum B$, where $SMexp(i)$ is the expected daily number of scent-marking events in enclosure area i; $B(i)$ is the number of daily behavioural scans recorded in enclosure area i; and $\sum SMobs$ and $\sum B$ are the total daily number of observed scent-marking events, and of behavioural scans, respectively.

To further investigate the social context of scent-marking behaviour, i.e. the influence of the identity of the nearest neighbour at the time of scent-marking, individual marking frequency for each potential pair of signaller–neighbour was compared in all five callitrichid groups. For

consistency, when individuals were alone in their enclosure section (i.e. out of sight from conspecifics) at the time of scent-marking, they were allocated the neighbour label 'None'. First, for each individual a Kruskal-Wallis rank sum test assessed the general influence of the factor 'identity of nearest neighbour' on hourly scent-marking frequency, followed by Dunn's post-hoc tests in order to investigate pairwise differences between potential neighbours. Individual signallers usually spent more time in proximity to certain conspecifics than others, which was likely to affect the scent-marking rate recorded for each pair of signaller–neighbour. Therefore, values of individual scent-marking frequencies per nearest neighbour were then weighted by dividing them by the proportion of time spent in closest proximity to each potential nearest neighbour during observations. This was measured as the hourly proportion of 2 min scans recorded when closest to each potential nearest neighbour. Second, a social network analysis approach (Croft, James and Krause, 2007) was applied to describe the observed variation in individual scent-marking activity across all potential nearest neighbours. Social network analysis in the study of animal behaviour was first introduced by Altmann (1968), who described the flow of social signals among members of a free-ranging population of rhesus macaques, *Macaca mulatta*, using sociograms, i.e. diagrams representing the relationships between each pair of individuals in a social group. More recently, sociograms have been used for example to assess intragroup affiliative behaviour between mother ring-tailed lemurs, *Lemur catta*, and their offspring (Nakamichi and Koyama, 2000); as well as to describe grooming behaviour in female hamadryas baboons, *Papio hamadryas* (Swedell, 2002; review by Krause, Croft and James, 2007). Here, sociograms were built using weight matrices of hourly scent-marking frequencies of all individual signallers given the identity of their nearest neighbour, weighted for the proportion of time each pair spent in proximity, for each callitrichid group studied (function qgraph() in R package 'qgraph'; Epskamp et al., 2012).

II.3. Results

II.3.1. Total scent-marking events recorded

A total of 847 individual scent-marking events were recorded across the 25 animals observed belonging to the five callitrichid groups studied. The number of scent-marking events recorded daily ranged from 4–50 per group (median 16.5 ±SD 8.94), and from 0–42 per individual (2 ±5.46), although there was no statistical difference in overall scent-marking activity between days of observation (Kruskal-Wallis rank sum test: χ^2= 12.032, *df*= 9, *P*= 0.212). This allowed all daily recordings, i.e. nine days for the silvery marmoset group, and ten days for the four tamarin groups, to be pooled into a single dataset.

II.3.2. Identity of the signaller: differences at the levels of species, group, sex, reproductive status, and the individual

Scent-marking frequency differed significantly between species (Kruskal-Wallis rank sum test: χ^2= 13.060, *df*= 2, *P*= 0.001), with silvery marmosets marking 3 ±3.18 times per hour (median ±SD), emperor tamarins 2.5 ±1.41 times per hour, and cotton-top tamarins 4 ±2.67 times per hour. Cotton-top tamarins scent-marked significantly more than both other species (**Fig. II.2a**; **Table II.3**). Scent-marking frequency also differed between study sites (χ^2= 37.748, *df*= 2, *P*< 0.001), with animals at DMP scent-marking significantly more frequently (3.5 ±2.44 marks per hour) than those at TZ (2.5 ±1.75 marks per hour) and PWP (3 ±2.25 marks per hour; **Table II.3**). Given the significant differences observed between species as well as study sites, it appeared important to consider each callitrichid group individually for the rest of the analyses. When considering each callitrichid group individually, there was an overall variation in scent-marking frequency across groups (χ^2= 20.359, *df*= 4, *P*< 0.001). In particular, the cotton-top tamarin group housed at DMP scent-marked the most frequently (4 ±4.35 marks per hour), which was significantly more frequent than the emperor tamarin groups at DMP (2 ±1.97 marks per hour) and TZ (3 ±1.96 marks per hour), and the silvery marmoset group at TZ (3 ±3.18 marks per hour; **Fig. II.2b**; **Table II.3**). The difference with the cotton-top tamarin group at PWP (3 ±2.29 marks per hour) was not significant (**Table II.3**).

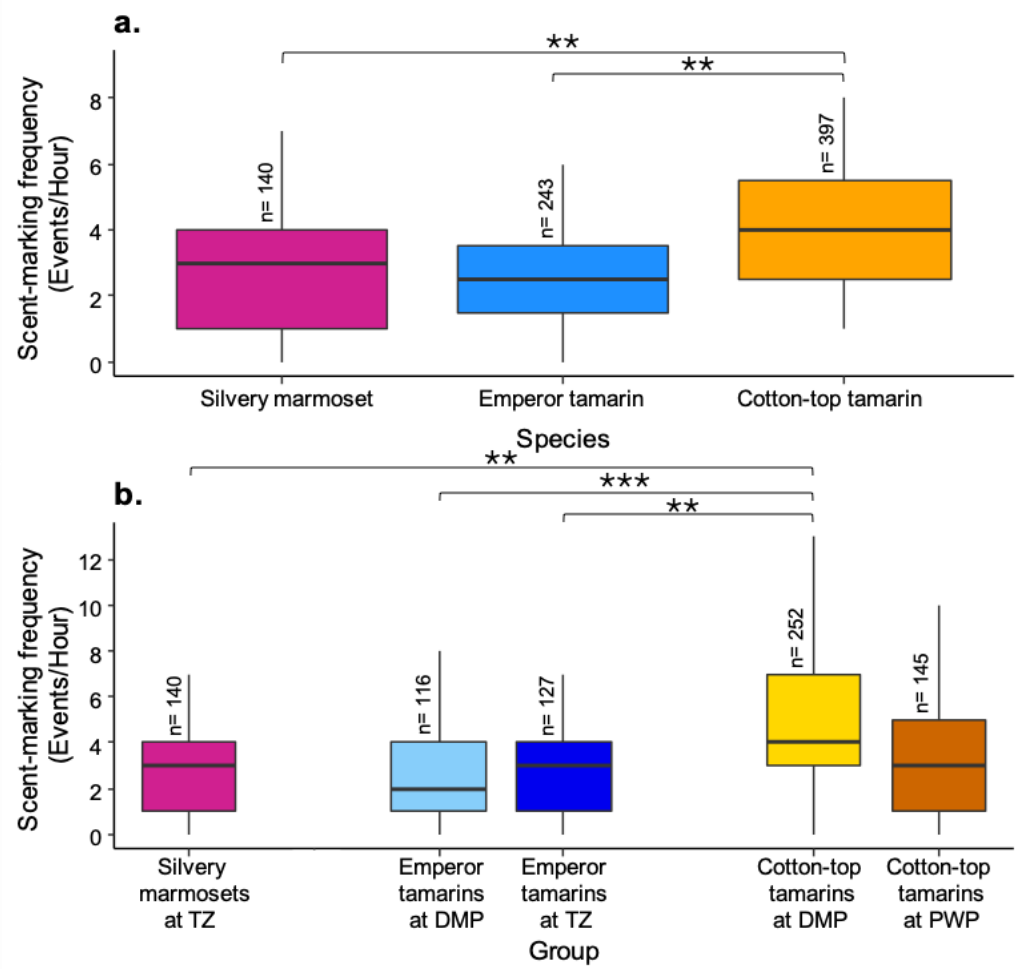


Figure II.2. Tukey's box-and-whiskers plots showing variation in scent-marking frequency between **a.** the three callitrichid species; and **b.** the five groups studied. Boxes indicate the median and interquartile range (IQR); whiskers give the smallest value ≥ lower hinge -1.5*IQR, and largest value ≤ upper hinge +1.5*IQR; n= sample size. Asterisks indicate statistically significant differences in pairwise Dunn's tests for categories of n>2 (★ $P \leq 0.05$, ★★ $P \leq 0.01$, ★★★ $P \leq 0.001$; **Table II.3**).

Table II.3. Results of Kruskal-Wallis rank sum tests of difference, and Dunn's post-hoc tests on pairwise comparisons, on scent-marking frequency (i.e. number of scent-marking events per one-hour observation bout), between species, study sites, and callitrichid groups. χ^2= χ^2-statistic; *df*= degrees of freedom; *Z*= *Z*-statistic; and *P*= p-value, significant at $P \leq 0.05$ (in bold).

Category tested	Kruskal-Wallis rank sum tests	Pairwise comparisons	Dunn's post-hoc tests
Species	χ^2= 13.060, *df*= 2, ***P*= 0.001**	Silvery marmosets – Emperor tamarins	*Z*= 0.020, *P*= 1.000
		Silvery marmosets – Cotton-top tamarins	*Z*= 3.119, ***P*= 0.005**
		Emperor tamarins – Cotton-top tamarins	*Z*= 3.140, ***P*= 0.005**
Study site	χ^2= 37.748, *df*= 2, ***P*< 0.001**	DMP – PWP	*Z*= 1.881, *P*= 0.180
		DMP – TZ	*Z*= 2.829, ***P*= 0.014**
		PWP – TZ	*Z*= 0.948, *P*= 1.000
Group	χ^2= 20.359, *df*= 4, ***P*< 0.001**	Silvery marmosets at TZ – Emperor tamarins at DMP	*Z*= 0.491, *P*= 1.000
		Silvery marmosets at TZ – Emperor tamarins at TZ	*Z*= 0.202, *P*= 1.000
		Silvery marmosets at TZ – Cotton-top tamarins at DMP	*Z*= 3.541, ***P*= 0.004**
		Silvery marmosets at TZ – Cotton-top tamarins at PWP	*Z*= 0.893, *P*= 1.000
		Emperor tamarins at DMP – Emperor tamarins at TZ	*Z*= 0.692, *P*= 1.000
		Emperor tamarins at DMP – Cotton-top tamarins at DMP	*Z*= 4.031, ***P*= 0.001**
		Emperor tamarins at DMP – Cotton-top tamarins at PWP	*Z*= 1.383, *P*= 1.000
		Emperor tamarins at TZ – Cotton-top tamarins at DMP	*Z*= 3.339, ***P*= 0.008**
		Emperor tamarins at TZ – Cotton-top tamarins at PWP	*Z*= 0.691, *P*= 1.000
		Cotton-top tamarins at DMP – Cotton-top tamarins at PWP	*Z*= 2.648, *P*= 0.081

There was an overall variation in scent-marking frequency at the level of sex (Kruskal-Wallis rank sum test: χ^2= 73.769, *df*= 1, *P*< 0.001) and reproductive status (χ^2= 40.321, *df*= 1, *P*< 0.001; **Table II.4**). However, when considering each callitrichid group individually, the differences observed were variable. Females scent-marked more than males, significant for both emperor tamarin groups and both cotton-top tamarin groups; however, this was not observed in the silvery marmoset group at TZ (**Fig. II.3a**; **Table II.4**). Non-reproductive individuals (i.e. juveniles and subordinates) scent-marked significantly more than the reproductive pair in the silvery marmoset group at TZ and the cotton-top tamarin group at PWP, whereas the reproductive pair of cotton-top tamarins at DMP scent-marked more often than the non-reproductive individuals (**Fig. II.3b**; **Table II.4**).

Table II.4. Results of Kruskal-Wallis rank sum tests of difference, and Dunn's post-hoc tests on pairwise comparisons, on scent-marking frequency (i.e. number of scent-marking events per one-hour observation bout), for each category tested: time of day, sex, reproductive status, individual, scent-marking type, scent-gland used, scent-marking duration, enclosure area, substrate chosen, presence of conspecifics, and investigative response. All tests are run on the full dataset (i.e. all callitrichid groups) as well as for each callitrichid group studied, for categories of sample size n> 2. χ^2= χ^2-statistic; *df*= degrees of freedom; *Z*= *Z*-statistic; and *P*= p-value, significant at *P*≤ 0.05 (in bold).

Category tested	**Kruskal-Wallis rank sum tests**	**Pairwise comparisons**	**Dunn's post-hoc tests**					
			All groups	**Silvery at TZ**	**Emperor at DMP**	**Emperor at TZ**	**Cotton-top at DMP**	**Cotton-top at PWP**
Sex	χ^2= 73.769, *df*= 1, ***P*< 0.001**	Male – Female	*Z*= 8.589, ***P*< 0.001**	*Z*= 0.957, *P*= 0.340	*Z*= 5.507, ***P*< 0.001**	*Z*= 2.734, ***P*= 0.006**	*Z*= 5.916, ***P*< 0.001**	*Z*= 5.820, ***P*< 0.001**
Reproductive status	χ^2= 40.321, *df*= 1, ***P*< 0.001**	Reproductive – Non-repro.	*Z*= 6.350, ***P*< 0.001**	*Z*= 2.467, ***P*= 0.014**		*Z*= 0.365, *P*= 0.710	*Z*= 8.043, ***P*< 0.001**	*Z*= 2.242, ***P*= 0.025**
Individual (1/2)	NA (unbalanced groups)	Repro. ♀ – Repro. ♂	*Z*= 7.896, ***P*< 0.001**	*Z*= 2.896, *P*= 0.079	*Z*= 5.507, ***P*< 0.001**	*Z*= 3.316, ***P*= 0.014**	*Z*= 6.849, ***P*< 0.001**	*Z*= 2.673, *P*= 0.113
		Repro. ♀ – Older subord. ♀	NA	*Z*= 0.642, *P*= 1.000				
		Repro. ♀ – Subord. ♀	*Z*= 8.372, ***P*< 0.001**	*Z*= 2.698, *P*= 0.146		*Z*= 2.335, *P*= 0.293		*Z*= 3.674, ***P*= 0.004**
		Repro. ♀ – Subord.♂	*Z*= 12.602, ***P*< 0.001**	*Z*= 2.304, *P*= 0.446		*Z*= 3.353, ***P*= 0.024**		*Z*= 3.935, ***P*= 0.001**
		Repro. ♀ – Juvenile ♀/♂	*Z*= 14.634, ***P*< 0.001**	*Z*= 0.593, *P*= 1.000		*Z*= 5.272, ***P*< 0.001**	*Z*= 8.785, ***P*< 0.001**	n too small
		Repro. ♀ – Juvenile ♂	*Z*= 14.467, ***P*< 0.001**	*Z*= 0.642, *P*= 1.000		*Z*= 5.650, ***P*< 0.001**	*Z*= 9.569, ***P*< 0.001**	*Z*= 4.679, ***P*< 0.001**
		Repro. ♂ – Older subord.♀	NA	*Z*= 3.538, ***P*= 0.009**				
		Repro. ♂ – Subord.♀	*Z*= 0.476, *P*= 1.000	*Z*= 0.198, *P*= 1.000		*Z*= 0.981, *P*= 1.000		*Z*= 6.347, ***P*< 0.001**

Table II.4. Continued (1/3).

Category tested	Kruskal-Wallis rank sum tests	Pairwise comparisons	Dunn's post-hoc tests					
			All groups	Silvery at TZ	Emperor at DMP	Emperor at TZ	Cotton-top at DMP	Cotton-top at PWP
Individual (2/2)	NA (unbalanced groups)	Repro. ♂ – Subord. ♂	*Z*= 4.706, ***P*< 0.001**	*Z*= 0.593, *P*= 1.000		*Z*= 0.163, *P*= 1.000		*Z*= 1.262, *P*= 1.000
		Repro. ♂ – Juvenile ♀/♂	*Z*= 6.739, ***P*< 0.001**	*Z*= 3.489, ***P*= 0.010**		*Z*= 1.956, *P*= 0.575	*Z*= 1.936, *P*= 0.317	n too small
		Repro. ♂ – Juvenile ♂	*Z*= 6.572, ***P*< 0.001**	*Z*= 2.254, *P*= 0.508		*Z*= 2.334, *P*= 0.294	*Z*= 2.720, ***P*= 0.039**	*Z*= 2.007, *P*= 0.672
		Older subord.♀ – Subord.♀	NA	*Z*= 3.340, ***P*= 0.018**				
		Older subord.♀ – Subord.♂	NA	*Z*= 2.946, *P*= 0.068				
		Older subord.♀ – Juvenile ♀/♂	NA	*Z*= 0.049, *P*= 1.000				
		Older subord.♀ – Juvenile ♂	NA	*Z*= 1.284, *P*= 1.000				
		Subord.♀ – Subord.♂	*Z*= 4.230, ***P*< 0.001**	*Z*= 0.395, *P*= 1.000		*Z*= 0.818, *P*= 1.000		*Z*= 7.609, ***P*< 0.001**
		Subord.♀ – Juvenile ♀/♂	*Z*= 6.262, ***P*< 0.001**	*Z*= 3.291, ***P*= 0.021**		*Z*= 2.937, ***P*= 0.050**		n too small
		Subord.♀ – Juvenile ♂	*Z*= 6.095, ***P*< 0.001**	*Z*= 0.836, *P*= 0.056		*Z*= 3.315, ***P*= 0.014**		*Z*= 8.354, ***P*< 0.001**
		Subord. ♂ – Juvenile ♀/♂	*Z*= 2.032, *P*= 0.884	*Z*= 2.896, *P*= 0.079		*Z*= 2.120, *P*= 0.511		n too small
		Subord. ♂ – Juvenile ♂	*Z*= 1.865, *P*= 1.000	*Z*= 1.661, *P*= 1.000		*Z*= 2.498, *P*= 0.188		*Z*= 0.745, *P*= 1.000
		Juvenile ♀/♂ – Juvenile ♂	*Z*= 0.167, *P*= 1.000	*Z*= 1.235, *P*= 1.000		*Z*= 0.378, *P*= 1.000	*Z*= 0.784, *P*= 1.000	n too small

Table II.4. Continued (2/3).

Category tested	Kruskal-Wallis rank sum tests	Pairwise comparisons	Dunn's post-hoc tests					
			All groups	Silvery at TZ	Emperor at DMP	Emperor at TZ	Cotton-top at DMP	Cotton-top at PWP
Scent-marking type	Silvery only: χ^2= 5.494, *df*= 1, **P= 0.019**	Gouge+secretions – Secretions or Urine+secretions	NA	*Z*= 2.344, **P= 0.019**				
	Tamarins only: χ^2= 141.920, *df*= 1, **P< 0.001**	Gouge+secretions – Secretions	NA	*Z*= 4.048, **P< 0.001**				
		Gouge+secretions – Urine+secretions	NA	*Z*= 6.061, **P< 0.001**				
		Urine+secretions – Secretions	*Z*= 11.763, **P< 0.001**	*Z*= 2.013, *P*= 0.132	*Z*= 4.344, **P< 0.001**	*Z*= 4.109, **P< 0.001**	*Z*= 7.667, **P< 0.001**	*Z*= 7.077, **P< 0.001**
Scent-gland	χ^2= 295.660, *df*= 2, **P< 0.001**	Anogenital – Suprapubic	*Z*= 10.645, **P< 0.001**	*Z*= 4.722, **P< 0.001**	*Z*= 7.058, **P< 0.001**	*Z*= 6.654, **P< 0.001**	*Z*= 8.440, **P< 0.001**	*Z*= 5.520, **P< 0.001**
		Anogenital – Sternal	*Z*= 17.017, **P< 0.001**	*Z*= 2.341, *P*= 0.058	n too small	*Z*= 8.443, **P< 0.001**	n too small	*Z*= 8.393, **P< 0.001**
		Suprapubic – Sternal	*Z*= 6.371, **P< 0.001**	*Z*= 7.063, **P< 0.001**	n too small	*Z*= 1.789, *P*= 0.220	n too small	*Z*= 2.873, **P= 0.012**
Scent-marking duration	χ^2= 59.844, *df*= 2, **P< 0.001**	Short – Medium	*Z*= 0.464, *P*= 1.000	*Z*= 6.594, **P< 0.001**	*Z*= 6.265, **P <0.001**	*Z*= 3.153, **P= 0.005**	*Z*= 6.818, **P< 0.001**	*Z*= 5.755, **P< 0.001**
		Medium – Long	*Z*= 6.455, **P< 0.001**	n too small	n too small	*Z*= 0.722, *P*= 1.000	*Z*= 7.950, **P< 0.001**	*Z*= 3.752, **P= 0.001**
		Short – Long	*Z*= 6.919, **P< 0.001**	n too small	n too small	*Z*= 2.431, **P= 0.045**	*Z*= 1.132, *P*= 0.770	*Z*= 2.003, *P*= 0.135

Table II.4. Continued (3/3).

Category tested	Kruskal-Wallis rank sum tests	Pairwise comparisons	Dunn's post-hoc tests					
			All groups	Silvery at TZ	Emperor at DMP	Emperor at TZ	Cotton-top at DMP	Cotton-top at PWP
Time of day	χ^2= 9.114, *df*= 2, ***P*= 0.010**	Morning – Midday	*Z*= 2.445, ***P*= 0.043**	*Z*= 0.436, *P*= 1.000	*Z*= 2.788, ***P*= 0.016**	*Z*= 2.171, *P*= 0.090	*Z*= 0.688, *P*= 1.000	*Z*= 0.909, *P*= 1.000
		Midday – Afternoon	*Z*= 0.311, *P*= 1.000	*Z*= 0.141, *P*= 1.000	*Z*= 0.296, *P*= 1.000	*Z*= 1.213, *P*= 0.675	*Z*= 1.452, *P*= 0.439	*Z*= 1.550, *P*= 0.360
		Morning – Afternoon	*Z*= 2.756, ***P*= 0.018**	*Z*= 0.295, *P*= 1.000	*Z*= 2.496, ***P*= 0.038**	*Z*= 3.384, ***P*= 0.002**	*Z*= 2.141, *P*= 0.097	*Z*= 0.640, *P*= 1.000
Enclosure area	χ^2= 280.630, *df*= 1, ***P*< 0.001**	Indoor – Outdoor	*Z*= 16.752, ***P*< 0.001**	n too small	n too small	*Z*= 3.969, ***P*< 0.001**	n too small	n too small
Substrate	χ^2= 330.220, *df*= 2, ***P*< 0.001**	Horizontal – Inclined	*Z*= 10.826, ***P*< 0.001**	*Z*= 0.854, *P*= 1.000	n too small	*Z*= 8.349, ***P*< 0.001**	*Z*= 0.208, *P*= 1.000	*Z*= 6.615, ***P*< 0.001**
		Horizontal – Vertical	*Z*= 18.053, ***P*< 0.001**	n too small	n too small	n too small	*Z*= 7.576, ***P*< 0.001**	*Z*= 8.674, ***P*< 0.001**
		Inclined – Vertical	*Z*= 7.227, ***P*< 0.001**	n too small	n too small	n too small	*Z*= 7.784, ***P*< 0.001**	*Z*= 2.058, *P*= 0.120
Presence of conspecifics	χ^2= 116.900, *df*= 1, ***P*< 0.001** NA (unbalanced groups)	None – ≥1	*Z*= 10.812, ***P*< 0.001**	*Z*= 4.340, ***P*< 0.001**	*Z*= 0.492, *P*= 0.620	*Z*= 5.585, ***P*< 0.001**	*Z*= 6.605, ***P*< 0.001**	*Z*= 7.732, ***P*< 0.001**
		None – One	*Z*= 0.631, *P*= 1.000	*Z*= 0.429, *P*= 1.000	*Z*= 0.492, *P*= 0.620	*Z*= 1.101, *P*= 0.810	*Z*= 2.909, ***P*= 0.011**	*Z*= 3.242, ***P*= 0.004**
		None – >1	*Z*= 6.818, ***P*< 0.001**	*Z*= 3.237, ***P*= 0.004**		*Z*= 5.617, ***P*< 0.001**	*Z*= 6.252, ***P*< 0.001**	*Z*= 6.824, ***P*< 0.001**
		One – >1	*Z*= 7.448, ***P*< 0.001**	*Z*= 2.808, ***P*= 0.015**		*Z*= 6.719, ***P*< 0.001**	*Z*= 9.160, ***P*< 0.001**	*Z*= 3.582, ***P*= 0.001**
Investigative response	χ^2= 314.040, *df*= 1, ***P*< 0.001**	Yes – No	*Z*= 17.721, ***P*< 0.001**	*Z*= 6.798, ***P*< 0.001**	n too small	*Z*= 7.411, ***P*< 0.001**	n too small	*Z*= 8.071, ***P*< 0.001**

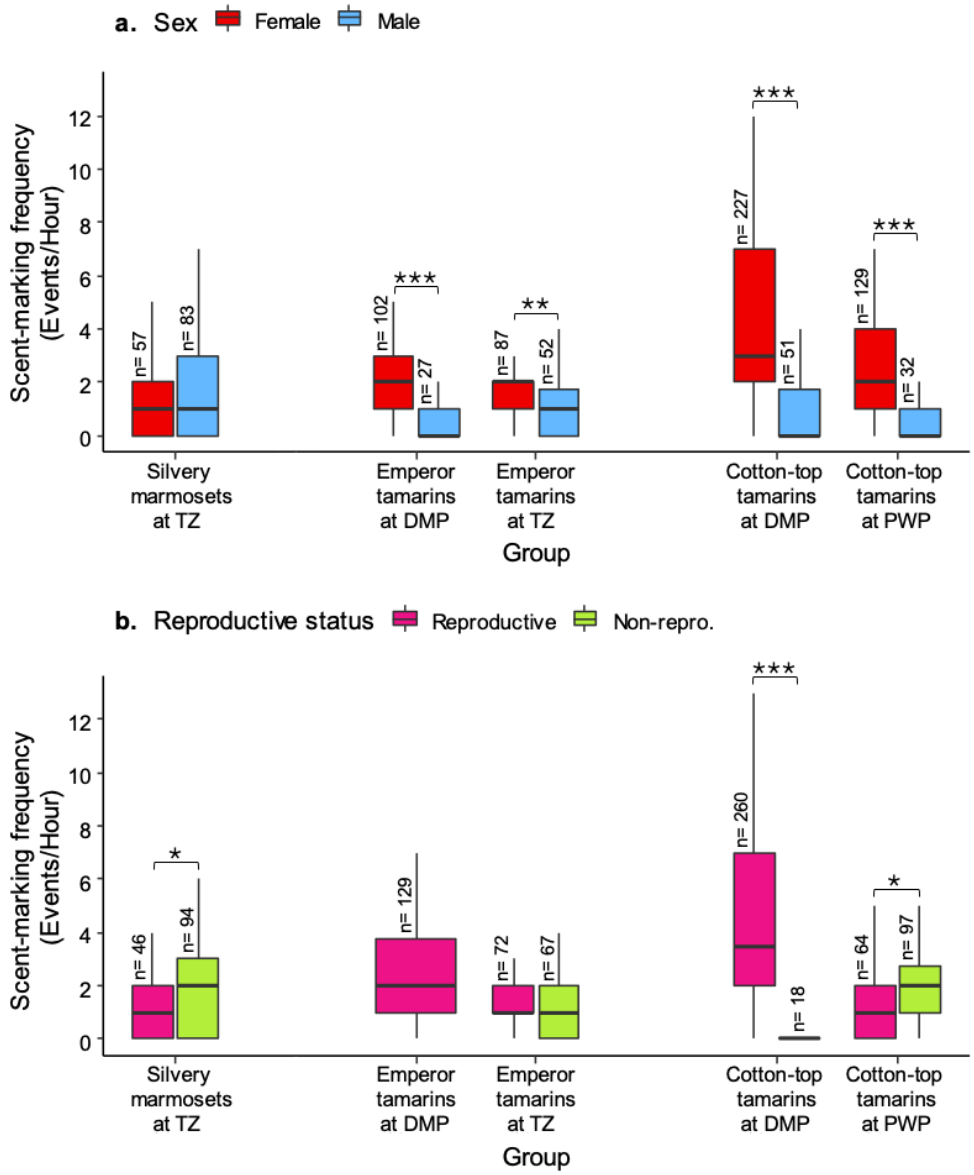


Figure II.3. Tukey's box-and-whiskers plots showing variation in scent-marking frequency between **a.** males and females; and **b.** reproductive and non-reproductive individuals, for each callitrichid group. Boxes indicate the median and interquartile range (IQR); whiskers give the smallest value ≥ lower hinge -1.5*IQR, and largest value ≤ upper hinge +1.5*IQR; n= sample size. Asterisks indicate statistically significant differences in pairwise Dunn's tests for categories of n> 2 (★ $P \leq 0.05$, ★★ $P \leq 0.01$, ★★★ $P \leq 0.001$; **Table II.4**).

In particular, the reproductive females in both emperor tamarin groups, and the cotton-top tamarin group at DMP, scent-marked more frequently than any of the other individuals (**Fig. II.4**; **Table II.4**). However, in cotton-top tamarins at PWP and silvery marmosets at TZ, the subordinate female scent-marked the most. Juveniles generally marked less than adults (**Fig. II.4**; **Table II.4**).

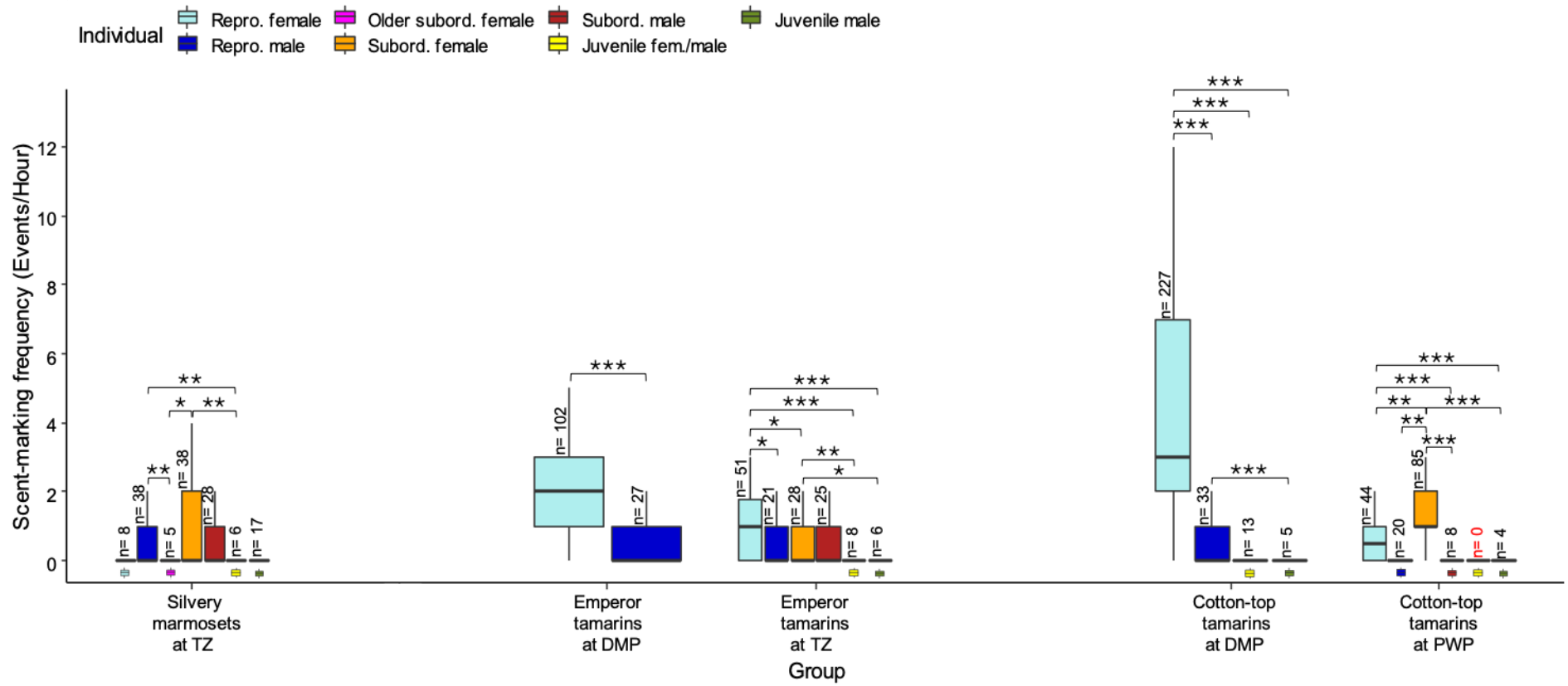


Figure II.4. Tukey's box-and-whiskers plots showing variation in scent-marking frequency between individuals for each callitrichid group. The Juvenile fem./male category (in yellow) corresponds to juvenile females in the silvery marmoset and emperor tamarin groups at TZ, and the cotton-top tamarin group at PWP, and to a juvenile male in the cotton-top tamarin group at DMP. Boxes indicate the median and interquartile range (IQR); whiskers give the smallest value ≥ lower hinge -1.5*IQR, and largest value ≤ upper hinge +1.5*IQR; n= sample size. Asterisks indicate statistically significant differences in pairwise Dunn's tests for categories of n> 2 (★ $P \leq 0.05$, ★★ $P \leq 0.01$, ★★★ $P \leq 0.001$; **Table II.4**).

II.3.3. Differences in scent-marking type, scent-gland use, and marking duration

A variety of scent-marking behaviour characteristics were observed during the study. First, callitrichids sometimes urinated just before depositing scent-marks, thus mixing glandular secretions with freshly voided urine, which potentially modified the scent signal produced. In addition, silvery marmosets at TZ often scent-marked immediately after gouging a hole in a wooden branch, thus covering with their secretions the exact spot they had just gouged. There was a significant variation in scent-marking types between simple glandular scent-marking and urine mixed with glandular secretions in the four tamarin groups studied (Kruskal-Wallis rank sum test: χ^2= 141.92, *df*= 1, *P*< 0.001); as well as between simple glandular scent-marking, urine mixed with glandular secretions, and scent-marking associated with tree-gouging in silvery marmosets (χ^2= 38.11, *df*= 2, *P*< 0.001; **Table II.4**). Tamarins, which do not gouge trees as part of their foraging behaviour, did not perform this scent-marking type. All tamarin groups scent-marked significantly more frequently without mixing secretions with urine; while in the silvery marmoset group at TZ scent-marking in association with tree-gouging was predominant (**Fig. II.5a**; **Table II.4**).

Second, callitrichids were observed to differentially use their three distinct scent-glands (i.e. anogenital, suprapubic, and sternal) to scent-mark (χ^2= 295.66, *df*= 2, *P*< 0.001; **Table II.4**). Silvery marmosets at TZ scent-marked mainly using their suprapubic gland; while tamarins from all four groups mainly used their anogenital gland (**Fig. II.5b**; **Table II.4**). The sternal gland was the most rarely used in all the groups studied.

Finally, the duration of scent-mark deposition was highly variable (i.e. short, medium, or long duration; χ^2= 59.844, *df*= 2, *P*< 0.001; **Table II.4**), thus potentially modifying the amount of secretion deposited at a given time. Most of the scent-marks produced by the groups of silvery marmosets and emperor tamarins at TZ, and the cotton-top group at PWP, were short; while both the emperor tamarins and cotton-top tamarins at DMP primarily produced medium duration scent-marks (**Fig. II.5c**; **Table II.4**).

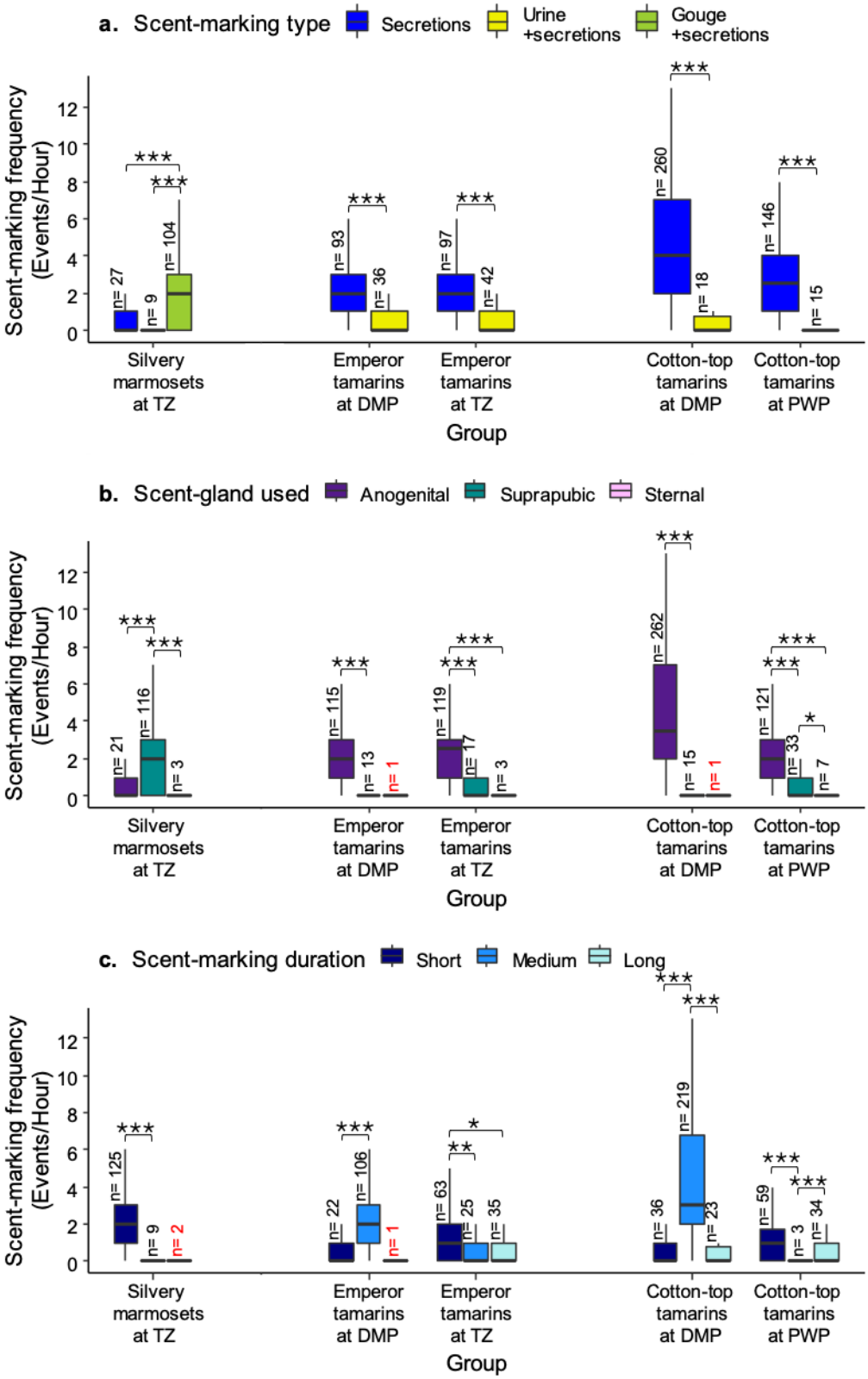


Figure II.5. Tukey's box-and-whiskers plots showing variation in scent-marking frequency with **a.** marking type, **b.** scent-gland used, and **c.** marking duration (i.e. short: 1–2 sec, medium: 3–6 sec, long: >6 sec), for each callitrichid group. Boxes indicate the median and interquartile range (IQR); whiskers give the smallest value ≥ lower hinge -1.5*IQR, and largest value ≤ upper hinge +1.5*IQR; n= sample size. Asterisks indicate statistically significant differences in pairwise Dunn's tests for categories of n> 2 (★ $P \leq 0.05$, ★★ $P \leq 0.01$, ★★★ $P \leq 0.001$; **Table II.4**).

II.3.4. Temporal and spatial differences in scent-marking activity

Scent-marking activity differed between time of day (χ^2= 9.114, *df*= 2, *P*= 0.010), where callitrichids marked less in the morning and more in the afternoon (**Table II.4**). When looking at differences between individual groups, this result was significant for emperor tamarins at DMP and TZ, while no difference was observed for silvery marmosets at TZ, and cotton-top tamarins at DMP and PWP (**Fig. II.6**; **Table II.4**).

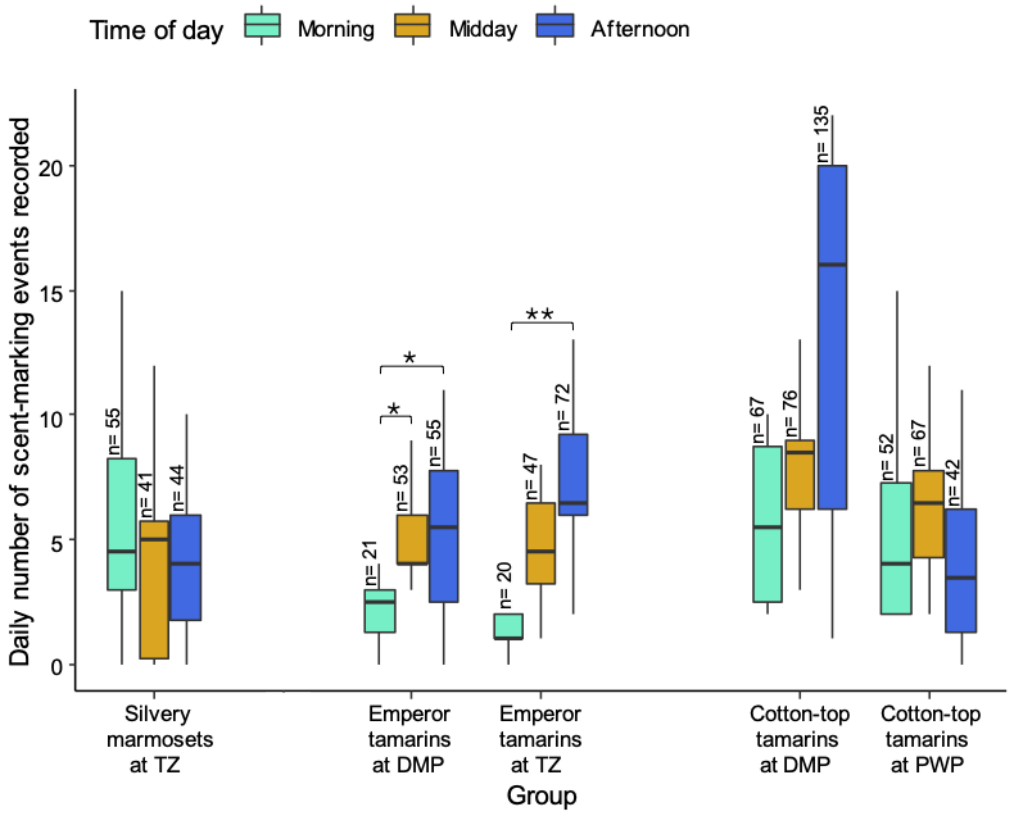


Figure II.6. Tukey's box-and-whiskers plots showing variation in the number of scent-marking events recorded at different times of day, i.e. in the morning (09:00–11:30), around midday (11:30–14:00), and in the afternoon (14:00–16:40), for each callitrichid group. Boxes indicate the median and interquartile range (IQR); whiskers give the smallest value ≥ lower hinge -1.5*IQR, and largest value ≤ upper hinge +1.5*IQR; n= sample size. Asterisks indicate statistically significant differences in pairwise Dunn's tests (★ *P*≤ 0.05, ★★ *P*≤ 0.01, ★★★ *P*≤ 0.001; **Table II.4**).

Scent-marking activity differed between enclosure areas used (i.e. indoor and outdoor areas; Kruskal-Wallis rank sum test: χ^2= 280.630, *df*= 1, *P*< 0.001), and the substrate chosen (i.e. horizontal, inclined, or vertical

branches; χ^2= 330.220, *df*= 2, *P*< 0.001; **Table II.4**). The emperor and cotton-top tamarin groups at DMP only scent-marked in their indoor enclosure areas (**Fig. II.7a**; **Table II.4**). Similarly, the silvery marmosets at TZ and cotton-top tamarins at PWP almost exclusively scent-marked indoors. Yet the emperor tamarins at TZ scent-marked outdoors 27.7% of the time (**Fig. II.7a**; **Table II.4**).

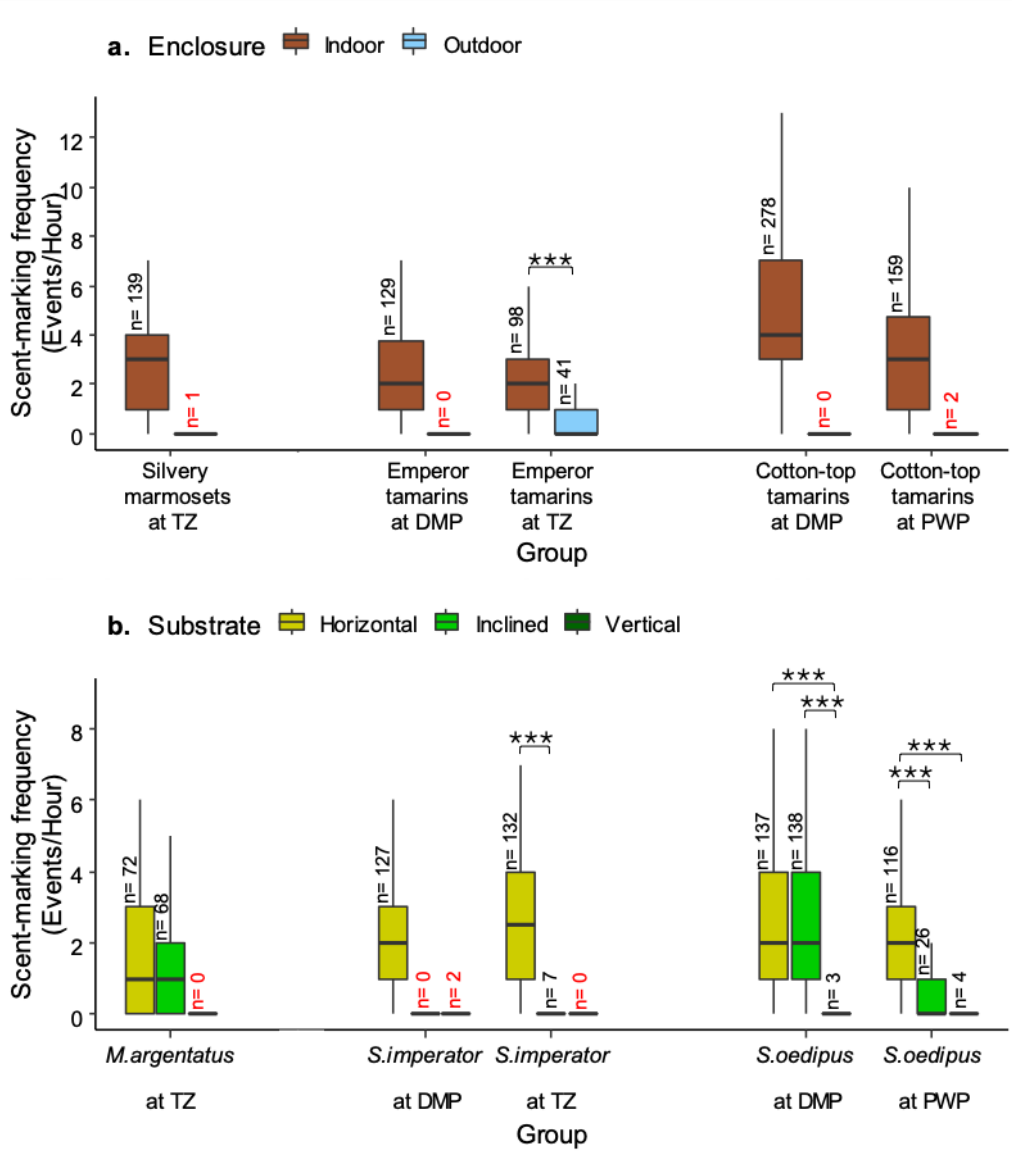


Figure II.7. Tukey's box-and-whiskers plots showing variation in scent-marking frequency between **a.** enclosure areas, and **b.** substrate chosen, for each callitrichid group. Boxes indicate the median and interquartile range (IQR); whiskers give the smallest value ≥ lower hinge -1.5*IQR, and largest value ≤ upper hinge +1.5*IQR; n= sample size. Asterisks indicate statistically significant differences in pairwise Dunn's tests for categories of n> 2 (✶ *P*≤ 0.05, ✶✶ *P*≤ 0.01, ✶✶✶ *P*≤ 0.001; **Table II.4**).

Nevertheless, this choice of enclosure area simply reflected the general use of space for all the groups, as indicated by non-significant Pearson's χ^2 tests of difference between observed and expected scent-marking frequencies in indoor and outdoor enclosure areas (**Table II.5**).

Table II.5. Results of Pearson's χ^2 tests with Yates' continuity correction, comparing mean daily observed scent-marking frequency in indoor and outdoor enclosure areas, with expected values given the general use of space recorded during observation, for each callitrichid group. SE= standard error of the mean; χ^2= χ^2-statistic; *df*= degrees of freedom; and *P*= p-value, significant at *P*≤ 0.05 (in bold).

Group	Enclosure area	Mean daily observed scent-marking frequency (±SE)	Mean daily expected scent-marking frequency (±SE)	Pearson's χ^2 test of difference
Silvery marmosets at TZ	Indoor	17.375 (±2.449)	16.346 (±0.627)	χ^2< 0.001, *df*= 1, *P*= 0.979
	Outdoor	0.125 (±0.125)	1.154 (±0.465)	
Emperor tamarins at DMP	Indoor	12.900 (±1.303)	11.551 (±0.283)	χ^2= 0.095, *df*= 1, *P*= 0.758
	Outdoor	0.000 (±0.000)	1.349 (±0.276)	
Emperor tamarins at TZ	Indoor	9.800 (±1.227)	9.974 (±0.747)	χ^2= 0, *df*= 1, *P*= 1.000
	Outdoor	4.100 (±0.912)	3.926 (±0.726)	
Cotton-top tamarins at DMP	Indoor	27.800 (±3.126)	27.641 (±0.131)	χ^2< 0.001, *df*= 1, *P*= 1.000
	Outdoor	0.000 (±0.000)	0.159 (±0.106)	
Cotton-top tamarins at PWP	Indoor	15.900 (±2.079)	14.764 (±0.665)	χ^2= 0.013, *df*= 1, *P*= 0.911
	Outdoor	0.200 (±0.133)	1.336 (±0.468)	

Horizontal and inclined substrates were the most used to scent-mark, used significantly more than vertical ones (**Table II.4**). Silvery marmosets at TZ and cotton-top tamarins at DMP used inclined substrates as much as horizontal ones; whereas in the other groups horizontal substrates were used more for scent-marking (**Fig. II.7b**; **Table II.4**).

II.3.5. Social context of scent-marking behaviour

II.3.5.1. Presence of conspecifics and investigatory response to scent-marks

Scent-marking generally occurred in the presence of at least one conspecific (Kruskal-Wallis rank sum test: χ^2= 116.90, *df*= 1, *P*< 0.001); except for the pair of emperor tamarins at DMP, which scent-marked as often alone as they did in the presence of each other (**Table II.4**). In addition, cotton-top tamarins at DMP scent-marked significantly more often on their own than in presence of a single conspecific; while the opposite was found for the cotton-top tamarin group at PWP (**Fig. II.8a**; **Table II.4**).

Very few scent-marks elicited a visible investigative response (i.e. sniffing, muzzle-rubbing or overmarking) to the scent-mark (Kruskal-Wallis rank sum test comparing presence/absence of response: χ^2= 314.04, *df*= 1, *P*< 0.001; **Fig II.8b**; **Table II.4**). Twelve occurrences of an investigative response were recorded in the emperor tamarin group at TZ (8.6% of scent-marks recorded), seven occurrences in the cotton-top tamarin group at PWP (4.4% of scent-marks); and only three times in the silvery marmoset group at TZ (2.1% of scent-marks), and once in both tamarin groups at DMP (0.8% and 0.4% of all scent-marks in emperor, and cotton-top tamarins, respectively).

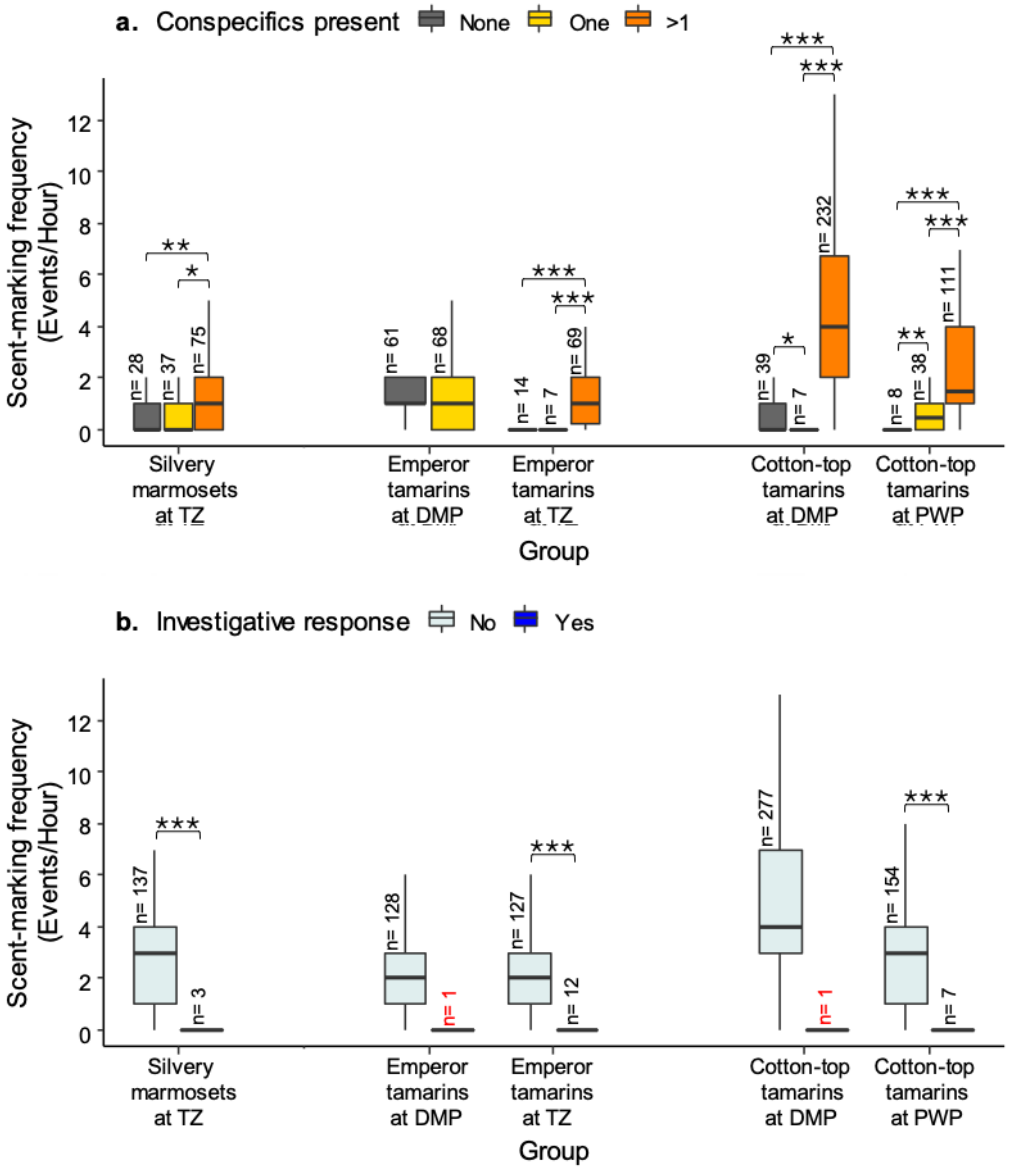


Figure II.8. Tukey's box-and-whiskers plots showing variation in scent-marking frequency with **a.** presence of conspecifics, and **b.** response to the scent-mark, for each callitrichid group. Boxes indicate the median and interquartile range (IQR); whiskers give the smallest value ≥ lower hinge -1.5*IQR and largest value ≤ upper hinge +1.5*IQR; n= sample size. Asterisks indicate statistically significant differences in pairwise Dunn's tests for categories of n> 2 (★ $P \leq 0.05$, ★★ $P \leq 0.01$, ★★★ $P \leq 0.001$; **Table II.4**).

Out of the 24 scent-marks that elicited a response, nine were deposited by reproductive females of both tamarin species (37.5%), 3 by the reproductive males (12.5%), 5 by the subordinate females (20.8%), 6 by the subordinate males (25%), and 1 by a juvenile female (4.2%). Identity of the responders varied between groups and individual signallers (**Table II.6**). Scent-marks from reproductive females were investigated mostly by reproductive and subordinate males; in the cotton-top tamarin group at PWP the subordinate and juvenile females also investigated the

reproductive female's marks. The reproductive male's scent-mark was only investigated by the reproductive female in the emperor tamarin group at TZ, and by the subordinate female in the cotton-top tamarin group at PWP. Individual responders to scent-marks from subordinate females varied between groups: in the silvery marmoset group at TZ the responder was the subordinate male, in the emperor tamarin group at TZ it was the reproductive male, while in the cotton-top tamarin group at PWP it was the reproductive female and the juvenile male. Scent-marks from the subordinate male emperor tamarin at TZ were investigated by the reproductive male and the subordinate female. Finally, the juvenile male silvery marmoset at TZ investigated a scent-mark from his twin sister (**Table II.6**).

Table II.6. Correspondence between individual signallers and responders for the scent-marks recorded to have elicited an investigatory response, across the five callitrichid groups studied. Numbers in brackets indicate the total number of scent-marks for which a response was recorded (N=24, although in four occurrences the responder was not identified).

Signallers	**Responders**				
	Silvery marmosets at TZ	Emperor tamarins at DMP	Emperor tamarins at TZ	Cotton-top tamarins at DMP	Cotton-top tamarins at PWP
Repro. ♀	NA	Repro.♂ (1)	Repro. ♂ (2) Subord. ♂ (1)	Repro. ♂ (1)	Subord. ♀ (1) Subord. ♂ (1) Juvenile ♀ (1)
Repro. ♂	NA	NA	Repro. ♀ (1)	NA	Subord. ♀ (2)
Subord. ♀	Subord. ♂ (1)		Repro. ♂ (1)		Repro. ♀ (1) Juvenile ♂ (1)
Subord. ♂	NA		Repro. ♂ (2) Subord. ♀ (2)		NA
Juvenile ♀	Juvenile ♂ (1)		NA	NA	NA

II.3.5.2. Individual variation in scent-marking activity with the identity of the nearest neighbour

Individual patterns of scent-marking were sometimes influenced by the identity of the nearest neighbour at the time of marking. After weighting individual values of scent-marking frequency for the proportion of time each pair of signaller–neighbour spent in closest proximity, sociograms were built to illustrate the relative distribution of individual scent-marking effort between potential nearest neighbours, for each callitrichid group. The emperor tamarin group at DMP was not included at this level of analysis because it was composed of two individuals only. The weighted hourly individual scent-marking frequencies ranged from 0–1.3 in the silvery marmoset group at TZ (see **Appendix B**, **Table B.1**); 0–1.1 in the emperor tamarin group at TZ (**Table B.2**); 0–3.2 in the cotton-top tamarin group at DMP (**Table B.3**); and 0–1.3 in the cotton-top tamarin group at PWP **Table B.4**).

In the silvery marmoset group at TZ, the subordinate male and female primarily scent-marked when they were alone (weighted hourly marking frequency = 1.267 and 0.916, respectively; **Fig. II.9**; see **Appendix B**, **Table B.1**). The reproductive male often marked next to the reproductive female (weighted hourly marking frequency = 0.912) and the juvenile female (weighted hourly marking frequency = 0.852); and the juvenile male next to the older subordinate female (weighted hourly marking frequency = 0.357), although this was not reciprocal. Similarly, the subordinate male and female marked more when next to every potential nearest neighbour than was reciprocal (**Fig. II.9**; **Table B.1**). The older subordinate female marked more next to the reproductive male (weighted hourly marking frequency = 0.278) than when she was alone (weighted hourly marking frequency = 0.126), which contrasted with the unweighted scent-marking frequency values. The reproductive female never marked next to the subordinate male or alone; the older subordinate female when next to any conspecific but the reproductive male; the subordinate female when next to the subordinate male or the infant; the juvenile female when next to the reproductive female, the older subordinate female, the subordinate male, the infant or alone; and the juvenile male when next to the subordinate female or alone (**Fig. II.9**; **Table B.1**).

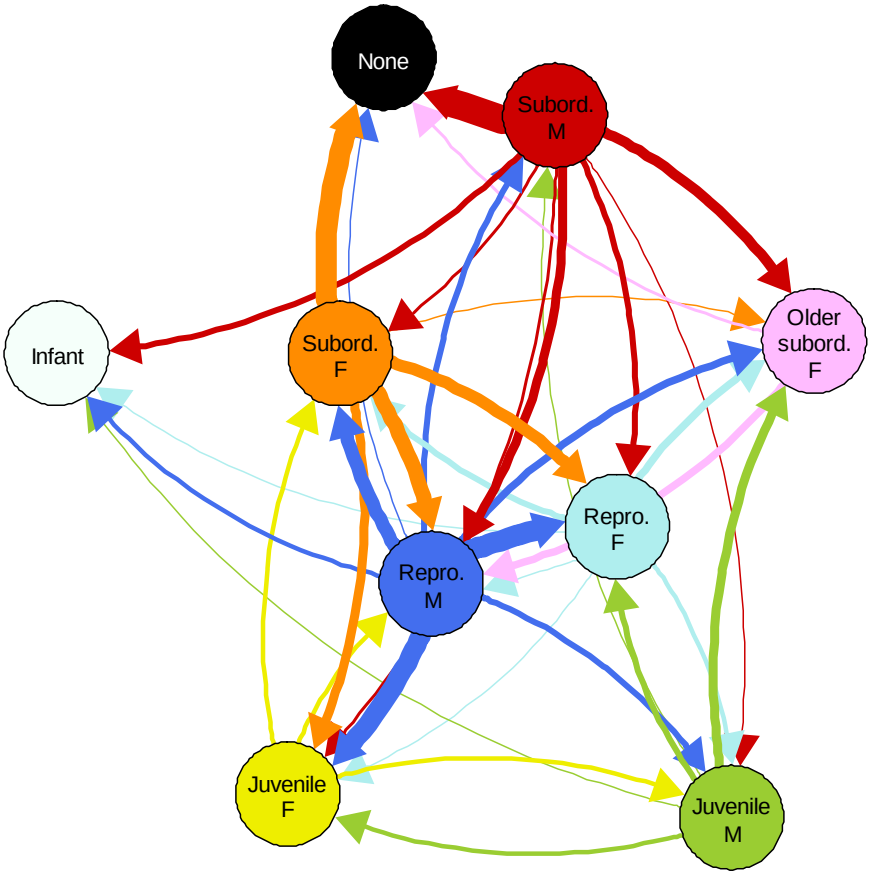


Figure II.9. Sociogram representing the relative distribution of scent-marking effort when in the presence of different nearest neighbours, for each individual silvery marmoset at TZ. Each node represents an individual, and directed arrows of matching colour indicate scent-marking frequency of this individual when next to each potential neighbour, weighted for the proportion of time the pair spent in proximity. Arrows' thickness is proportional to weighted scent-marking frequency for each pair of signaller–neighbour (see **Appendix B**, **Table B.1**).

In the emperor tamarin group at TZ, the reproductive male and the subordinate male scent-marked preferentially when next to each other (weighted hourly marking frequency = 0.317 and 1.064, respectively; **Fig. II.10**; see **Appendix B**, **Table B.2**). Moreover, the reproductive female mainly marked next to the juvenile female (weighted hourly marking frequency = 0.983) and the reproductive male (weighted hourly marking frequency = 0.596); the subordinate female marked more next to the reproductive male (weighted hourly marking frequency = 0.425), the reproductive female (weighted hourly marking frequency = 0.399), and the juvenile male (weighted hourly marking frequency = 0.397), although this was not reciprocal. The reproductive male and the subordinate male never marked next to the reproductive female; the juvenile female next to the subordinate female or alone, and the juvenile male next to the subordinate male or alone (**Fig. II.10**; **Table B.2**).

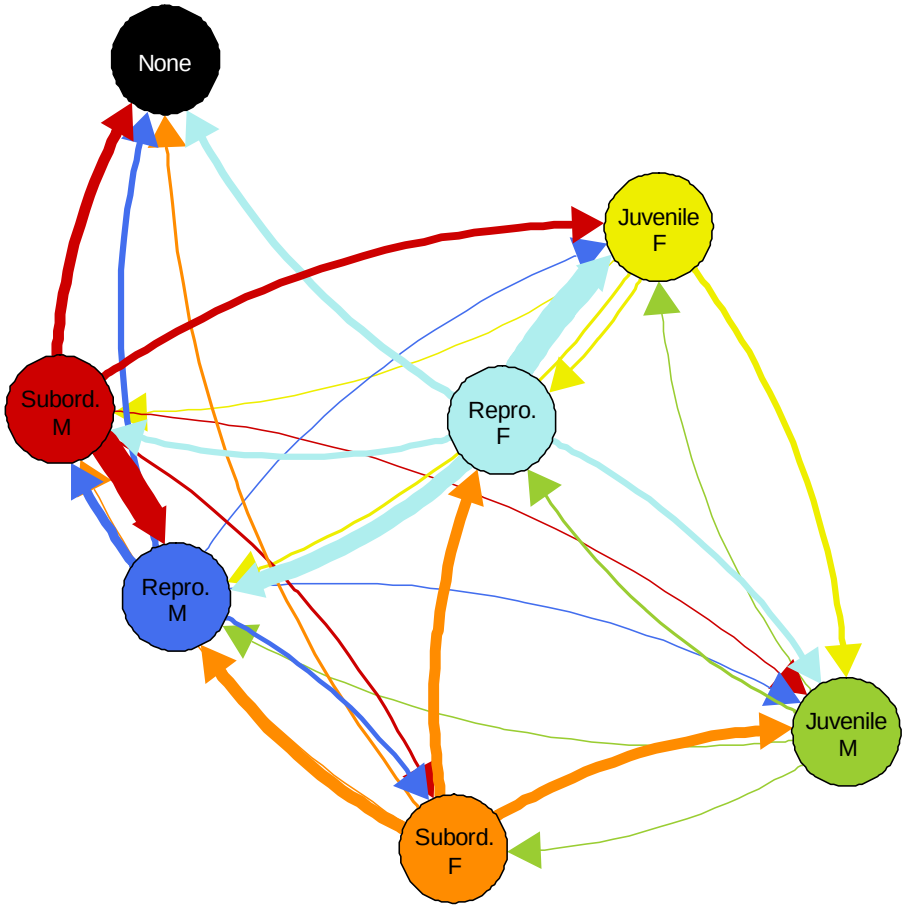


Figure II.10. Sociogram representing the relative distribution of scent-marking effort when in the presence of different nearest neighbours, for each individual emperor tamarin at TZ. Each node represents an individual, and directed arrows of matching colour indicate scent-marking frequency of this individual when next to each potential neighbour, weighted for the proportion of time the pair spent in proximity. Arrows' thickness is proportional to weighted scent-marking frequency for each pair of signaller–neighbour (see **Appendix B**, **Table B.2**).

In the cotton-top tamarin group at DMP, the reproductive female mainly scent-marked next to the reproductive male and the juvenile male M1 (weighted hourly marking frequency = 3.215 and 3.210, respectively; **Fig. II.11**; see **Appendix B**, **Table B.3**). The reproductive male marked more next to either of the juveniles (weighted hourly marking frequency for M1= 0.288; M2= 0.205) than when he was alone, and he never marked next to the reproductive female. Neither of the juveniles marked when they were alone in the enclosure section (**Fig. II.11**; **Table B.3**).

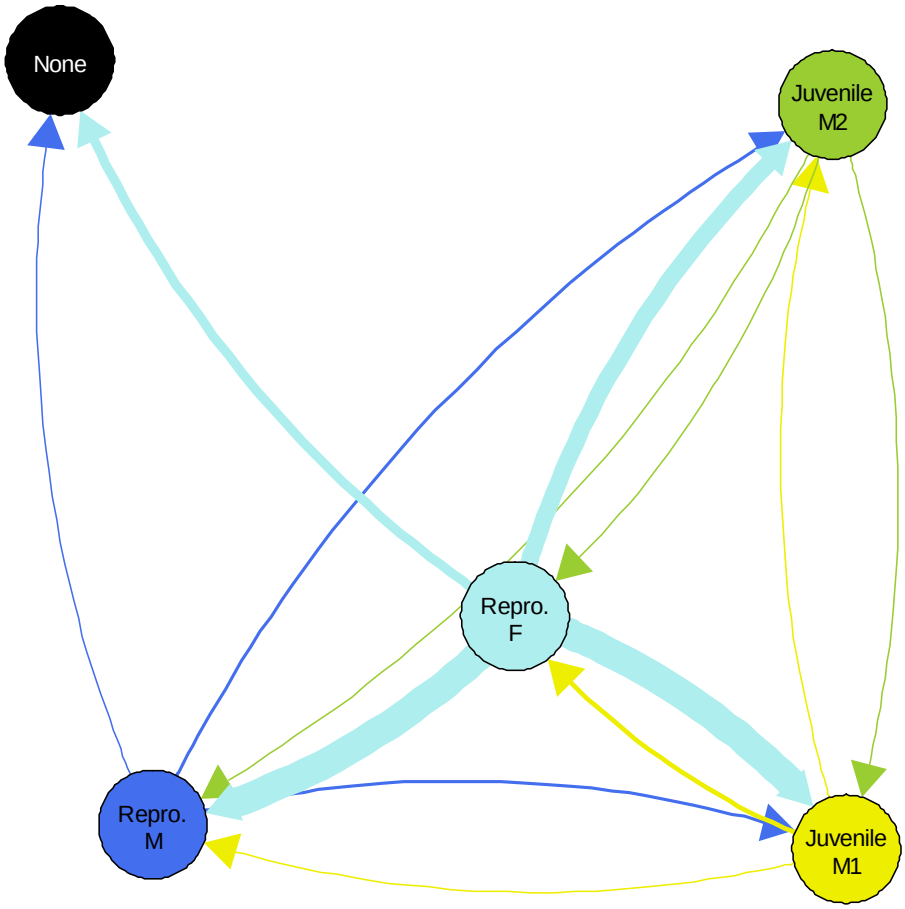


Figure II.11. Sociogram representing the relative distribution of scent-marking effort when in the presence of different nearest neighbours, for each individual cotton-top tamarin at DMP. Each node represents an individual, and directed arrows of matching colour indicate scent-marking frequency of this individual when next to each potential neighbour, weighted for the proportion of time the pair spent in proximity. Arrows' thickness is proportional to weighted scent-marking frequency for each pair of signaller–neighbour (see **Appendix B**, **Table B.3**).

Finally, in the cotton-top tamarin group at PWP, the reproductive female scent-marked mainly next to the subordinate male and the reproductive male (weighted hourly marking frequency = 1.347 and 1.052, respectively; **Fig. II.12**; see **Appendix B**, **Table B.4**). This was not reciprocal, as the subordinate male marked more next to the reproductive male (weighted hourly marking frequency = 0.123) and the juvenile female (weighted hourly marking frequency = 0.127); and the reproductive male marked more next to both juveniles (weighted hourly marking frequency = 0.318 and 0.349, respectively). Similarly, the subordinate female marked more when next to the subordinate male (weighted hourly marking frequency = 1.218) or the reproductive male (weighted hourly marking frequency = 0.965), which was not reciprocal. The reproductive male was never observed marking when next to the subadult male; the subordinate male when he was alone; and the juvenile male

when next to the reproductive male, the juvenile female or alone. The juvenile female did not scent-mark at all (**Fig. II.12**; **Table B.4**).

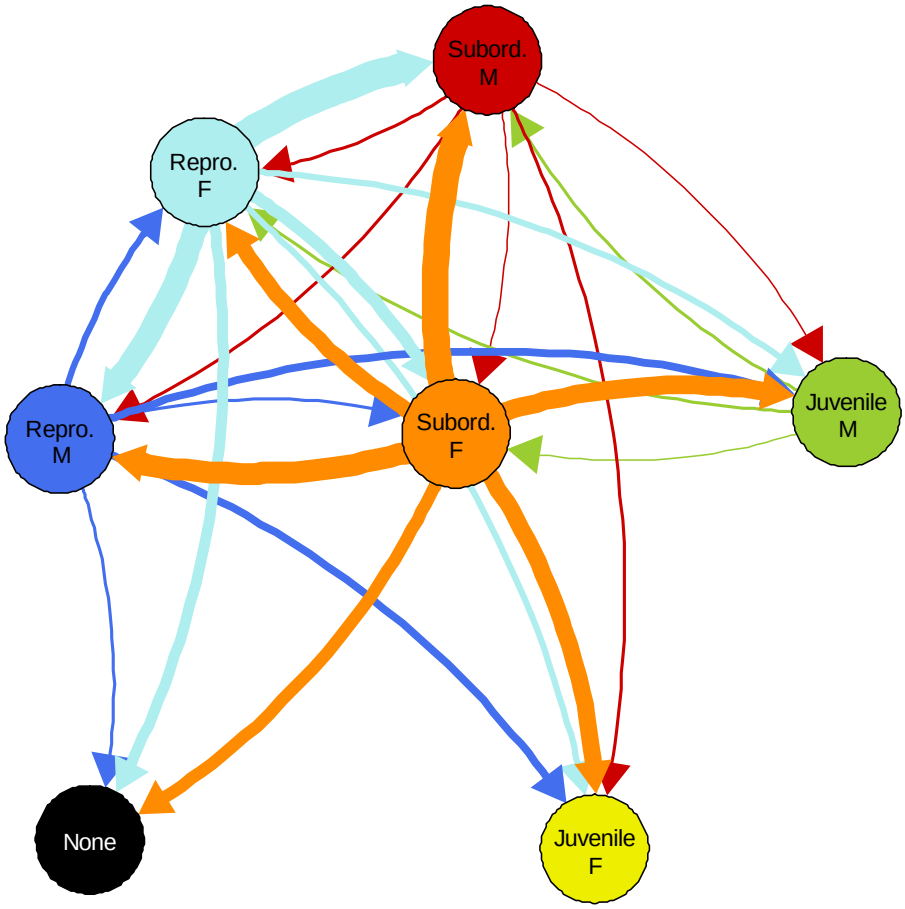


Figure II.12. Sociogram representing the relative distribution of scent-marking effort when in the presence of different nearest neighbours, for each individual cotton-top tamarin at PWP. Each node represents an individual, and directed arrows of matching colour indicate scent-marking frequency of this individual when next to each potential neighbour, weighted for the proportion of time the pair spent in proximity. Arrows' thickness is proportional to weighted scent-marking frequency for each pair of signaller–neighbour (see **Appendix B**, **Table B.4**).

II.4. Discussion

II.4.1. Characteristics of scent-marking behaviour in captive callitrichids

II.4.1.1. Patterns of identity in scent-marking activity at the levels of species, group, sex, reproductive status, and the individual

Results from this behavioural study showed that scent-marking activity differed between species and study sites, and further revealed variation between groups. Hourly scent-marking frequencies for silvery marmosets, emperor tamarins, and cotton-top tamarins, were 3 ±3.18, 2.5 ±1.41, and 4 ±2.67 marks per hour (median ±SD), respectively. Differences in scent-marking frequencies have also been observed between wild sympatric species. For example, Smith (1997) and Heymann (2001) found that Geoffroy's saddleback tamarins scent-marked more than sympatric moustached tamarins. Similarly, Watsa (pers. comm.) recorded a higher rate of scent-marking behaviour in Weddell's saddleback tamarins, *Leontocebus weddelli*, than in sympatric emperor tamarins. In addition, Koprowski (1993) found higher rates of cheek-rubbing in wild fox squirrels, *Sciurus niger*, than in sympatric eastern grey squirrels, *S. carolinensis*. These field observations support the hypothesis that characteristics of the species might influence scent-marking activity more than potential ecological characteristics, which could explain the interspecific differences found in the present study.

In comparison to the rates found in the present study, a much higher scent-marking frequency has been reported in laboratory-kept cotton-top tamarin pairs (ca. 25 marks per hour; French and Snowdon, 1981), common marmoset groups (30 marks per hour; Epple, 1970), and female red-bellied tamarins, *S. labiatus* (13 marks per hour; Coates and Poole, 1983). However, Smith and Gordon (2002) reported a mean of 1.88 marks per hour in three captive pairs of red-bellied tamarins. Furthermore, other studies on wild callitrichids have generally found similar lower hourly frequencies (i.e. 2.30 ±0.41 marks per hour in golden lion tamarins, Miller, Laszlo and Dietz, 2003; 2.06 marks per hour in common marmosets, Lazaro-Perea, Snowdon and Arruda, 1999; 0.52 marks per hour in moustached tamarins and 5.59 marks per hour in Geoffroy's saddleback tamarins, Heymann, 2001). Such variation between captive and wild studies might be explained by the differences in home range size, with

greater distances to travel between scent-marking locations in the wild compared with captivity, thus reducing the rate of marking. Alternatively, they could simply be an artefact of the fact that visibility of the animals' behaviour is poorer in wild conditions, in which a number of the deposited scent-marks may fail to be recorded. In the present study, visibility was enhanced by the captive environment. Nevertheless, enclosures were divided into several areas separated by walls or mesh, which made it impossible for a single observer to keep sight of all animals at all times; therefore whilst we expect the proportion of scent-marking successfully recorded to be greater than in wild conditions, it may be lower than in laboratory cages. Furthermore, general visibility of the animals varied between sites in this study. Tamarins at DMP were generally more visible than at the other sites, because the main enclosure areas were only separated by mesh, covered at places by the enclosure's furnishing, instead of walls, or even tunnels (i.e. between indoor and outdoor spaces), which might explain why observed scent-marking frequency at this site (i.e. 3.5 ±2.44 marks per hour) was greater than at TZ (2.5 ±1.75) and PWP (3 ±2.25). In addition, differences in enclosure size and furnishing, as well as husbandry procedures (e.g. number of daily feeding and enrichment sessions; Wormell et al., 2012) may have further led to differences in scent-marking behaviour between sites.

A conservative way to account for potential differences between species and sites was therefore to consider each callitrichid group individually. The callitrichid groups studied differed in size and composition, ranging from a pair of reproductive adults alone (i.e. emperor tamarins at DMP), to eight individuals including a reproductive pair and three generations of offspring (i.e. silvery marmosets at TZ), which may have further influenced scent-marking activity in this study. Nevertheless, the frequency of scent-marking behaviour recorded in this study was not proportional to group size, as the cotton-top tamarin group housed at DMP, composed of an reproductive pair and juvenile twins, scent-marked the most (4 ±4.35 marks per hour), whereas emperor tamarin groups at DMP and TZ, composed of a single reproductive pair, and an reproductive pair with two generations of offspring (N=6), respectively, scent-marked the least frequently (2 ±1.97, and 3 ±1.96 marks per hour). Intergroup variation in scent-marking frequency was also observed by Heymann (2001; range of hourly group scent-marking: 0.36–0.68, N=3 groups) and Huck et al.

(2004; range= 0.18–0.47 marks per hour, N=2) in wild moustached tamarins, Lazaro-Perea et al. (1999) in wild common marmosets (range= 1.14–2.25 marks per hour, N=5), and Epple (1970) in laboratory-kept common marmosets (range= 27.13–34.85 marks per hour, N=3). Various aspects of group composition will be further inspected in the following paragraphs.

Tamarin females scent-marked more frequently than males overall. A number of studies have found the same result in various callitrichid species in captivity (e.g. in cotton-top tamarins, Epple, Kuderling and Belcher, 1988; French and Snowdon, 1981; saddleback tamarins, French and Snowdon, 1981; red-bellied tamarins, Coates and Poole, 1983; Smith and Gordon, 2002; golden-headed lion tamarins, *Leontopithecus chrysomelas*, De Vleeschouwer et al., 2000; and common marmosets, Epple, 1970, 1972), as well as in the wild (e.g. in moustached tamarins, Heymann, 1998; and golden lion tamarins, Miller, Laszlo and Dietz, 2003). However, Lazaro-Perea, Snowdon and Arruda (1999) found no difference in scent-marking frequency between male and female wild common marmosets, nor did Oliveira and Macedo (2010) in black-tufted marmosets, *C. penicillata*, or Wolovich and Evans (2007) in captive pairs of another New World primate, the owl monkey, *Aotus nancymaae*. Anogenital and suprapubic scent-glands of callitrichids have been reported to be larger in females than males (first reported in Perkins, 1975; in cotton-top tamarins, French and Cleveland, 1984; and saddleback tamarins, Watsa, 2013; Zeller et al., 1988). This observation is in accordance with a reproductive function of scent-marking behaviour. Unlike males, female reproductive state varies cyclically with ovulation. While many female primates provide visual and/or acoustic cues of ovulation (e.g. sexual swellings in female mandrills, *Mandrillus sphinx*, Setchell, 2016; mating calls in female Barbary macaques, *Macaca sylvanus*, Pfefferle et al., 2008a; b), in female callitrichids ovulation is concealed (Dixson, 2012; Ziegler et al., 1993). Although female callitrichids engage in sexual behaviour throughout their reproductive cycle, which lasts 23 days in tamarins (French, Abbott and Snowdon, 1984), several studies have shown an increase of male sexual activity in the female periovulatory period (e.g. Smith and Abbott, 1998; Ziegler et al., 2005). Female callitrichids hence communicate their reproductive state to their pair-bonded mate, or other potential mates, via odour cues (e.g. in cotton-top tamarins, Ziegler et al., 1993; pygmy

marmosets, *Cebuella pygmaea*, Converse et al., 1995; and golden-headed lion tamarins, De Vleeschouwer et al., 2000). Similar studies of other primate taxa have further demonstrated the importance of odour cues in indicating female reproductive state (e.g. in Coquerel's sifakas, *Propithecus coquereli*, Greene and Drea, 2014; olive baboons, *Papio anubis*, Rigaill et al., 2013; and even humans, *Homo sapiens sapiens*, Haselton and Gildersleeve, 2016). Nevertheless, in the present study male silvery marmosets at TZ scent-marked more than females; indeed, in many mammalian species males scent-mark more frequently than females, primarily to signal territory and/or dominance (Albone and Shirley, 1984; Wyatt, 2014a). In wild callitrichids in particular, it was suggested that scent-marking by males may be a means of chemical mate guarding (Huck, Löttker and Heymann, 2004; Lledo-Ferrer, Peláez and Heymann, 2010; Manson, 1997). In addition, male odours are also known to trigger ovulation in young adult females, a phenomenon called the 'Vandenbergh effect' (Vandenbergh, 1969), particularly studied in the house mouse, *Mus musculus* (reviewed in Petrulis, 2013).

There was a significant effect of reproductive status on scent-marking behaviour overall, although this varied between groups, and appeared highly dependent on the presence of subordinate individuals in the groups. In both groups of emperor tamarins as well as the cotton-top tamarin group at DMP, the reproductive female scent-marked the most. This was in accordance with a role of scent-marking in advertisement of female reproductive state. However, in the cotton-top tamarin group at PWP and the silvery marmoset group at TZ, the subordinate female scent-marked more than the reproductive female. Callitrichids are cooperative breeders, which means that the dominant female is usually the only one to reproduce, and all group members participate in the raising of the young (Garber et al., 2016; Huck, Löttker and Heymann, 2004; Rylands and Mittermeier, 2013). Instead of reproducing themselves, subordinate females in their natal groups usually assist in the care of the infants. Reproductive suppression in subordinate females, usually the daughters of the dominant female, has been shown to occur through both behaviour and chemical cues from the dominant female (e.g. in common marmosets, Abbott et al., 1998; Barrett, Abbott and George, 1990; Saltzman et al., 1997; Ziegler and Sousa, 2002; Ziegler, 2013; cotton-top tamarins, Heistermann et al., 1989; Savage, Ziegler and Snowdon, 1988; and pygmy

marmosets, Spurlock, 2001; reviewed in Beehner and Lu, 2013). Common marmoset daughters experimentally separated from their mother rapidly start ovulating, yet the onset of ovulation is delayed if they are kept within scent contact of their mother (Barrett, Abbott and George, 1990; Saltzman et al., 1997), emphasizing the importance of chemosignals in callitrichid reproductive suppression. Yet several field studies have reported occurrences of multiple breeding females in groups of tamarins (e.g. in cotton-top tamarins, Savage et al., 1996; Geoffroy's saddleback tamarins, Calegaro-Marques, Bicca-Marques and Azevedo, 1995; and moustached tamarins, Smith et al., 2001a). Lazaro-Perea (2001), and Sousa et al. (2005; see also Arruda et al., 2005), suggested that various reproductive strategies may alternatively be used by subordinate common marmoset females: **1.** subordinate females may stay in their natal group as non-reproductive members for a certain period of time while waiting for a more favourable opportunity to reproduce, e.g. if the reproductive female dies or emigrates; they might at the same time engage in intergroup copulations with neighbouring males as a way to scan for vacancies for reproductive positions. Alternatively, **2.** subordinate females may attempt to reproduce, which can be successful (Smith et al., 2001a), but involves the risk of infanticide on their offspring. Finally, **3.** subordinate females may emigrate from their natal group when they reach reproductive maturity and establish a new group with neighbouring males. While scent-marking is used by dominant females to impose reproductive suppression on subordinate females; subordinate females, on the other hand, may use scent-marking as a way of advertising their reproductive state to the available males within their group, or to males of neighbouring groups (Heymann, 2006a; Lazaro-Perea, 2001; Lledo-Ferrer, Peláez and Heymann, 2011; Ziegler and Sousa, 2002). As a result, it is possible that the differences observed in the present study reflect different levels of female intrasexual competition for access to reproduction. Nevertheless, the exact mechanisms in place, as well as the overall variation between callitrichid species and between existing socio-sexual organizations both in the wild and in captivity, are yet to be described (Beehner and Lu, 2013).

The case of the silvery marmoset group in this study was particularly interesting: the reproductive female scent-marked very infrequently, which was likely owing to the fact that she was still nursing her last-born offspring at the time of the study. The older subordinate female

showed an even lower tendency to scent-mark; she was also the primary carer for the infant, besides the mother, and showed a very low aggression rate towards the other group members (pers. obs.). Hence, she displayed a typical subordinate helper behaviour. The younger subordinate female, on the other hand, scent-marked much more frequently, and showed more aggressive behaviour. It is plausible that this subordinate female, as she was becoming reproductively mature, was starting to challenge the reproductive female for dominance. The same can be hypothesized for the subordinate female cotton-top tamarin at PWP, which scent-marked more frequently than any other individuals in the group. In captivity, natural dispersal of young adult females from their natal group is of course impossible. Besides the risk of aggression from and towards animals that would have left their natal group should they have been in natural conditions, there is the risk of inbreeding if offspring reproduce with their parents. Increasing aggression between parents and older offspring is closely monitored by zookeepers, and individuals reaching sexual maturity are usually removed from their natal group and transferred to other zoos if they start showing exaggerated aggression (Twycross Zoo, pers. comm.). Juveniles generally scent-marked less frequently than adults and subordinates (no scent-marking was recorded for the female juvenile cotton-top tamarin at PWP), which further suggests an important role of scent-marking in reproduction and intrasexual competition in sexually mature animals.

II.4.1.2. Characteristics of scent-marking activity: differences in scent-mark type and duration, and scent-gland used

Scent-marks recorded in this study differed in type and duration. Callitrichids used pure glandular secretions as scent-marks more often than secretions mixed with urine. This was particularly noticeable for both groups of cotton-top tamarins. Voided urine is a known olfactory cue in mammals (Albone and Shirley, 1984; Burger, 2005; Goodwin et al., 2006), including primates (Colquhoun, 2011; DelBarco-Trillo et al., 2011, 2013; Osada et al., 2008; Palagi, Dapporto and Borgognini Tarli, 2005; Palagi and Norscia, 2009; Rigaill et al., 2017). Moreover, some primates such as squirrel monkeys, *Saimiri* spp., perform urine-marking by urinating on their hands, then rubbing them onto a substrate (Laska and Hudson, 1995), in a

similar fashion to glandular scent-marking. In callitrichids, pools of voided urine are often investigated by conspecifics (Epple et al., 1981; pers. obs.); and Heymann (2001) observed scent-marking accompanied by urination in wild moustached and Geoffroy's saddleback tamarins. By adjusting the proportion of the two fluids in their scent-marks, callitrichids may be able to convey different types of signal. In **Chapter 3**, I will assess whether the chemical composition of glandular secretions, deposited scent-marks, and urine differs, which would further support the idea of different signals, and potentially different functions, carried by various olfactory cues.

Silvery marmosets at TZ often combined scent-marking with tree-gouging, where they carved a hole in the enclosure wooden furniture using their strong specialized incisors, and scent-marked the spot just after; or they alternated gouging and scent-marking. Such behaviour has been observed in this species by Omedes (1981), and in other marmoset species (e.g. black-tufted marmosets, Lacher et al., 1981; and Aripuaña marmosets, *Mico intermedius* (formerly *Callithrix humeralifer intermedius*, Rylands, 1990). Marmosets naturally feed on gum and other tree exudates, which they stimulate the flow of by gouging holes through the bark to the cambium layer (Rylands, 1984). Tamarins occasionally feed on tree exudates, however they lack the dental adaptations to gouge trees (Rosenberger, 1978; Rylands and Mittermeier, 2013; Smith and Smith, 2013). The fact that silvery marmosets at TZ performed scent-marking while doing this foraging activity may support the hypothesis that scent-marking plays a role in food resource signalling, as was suggested by Thompson et al. (2018) in captive common marmosets. However, in captivity the branches gouged are dry, and therefore do not provide any food resource. Alternatively, silvery marmosets may favour newly gouged branch areas to scent-mark simply because the irregular surface absorbs and/or retains better the deposited secretions, as has been suggested by Rylands (1984, 1990).

Tamarins of both species preferentially used their anogenital scent-gland when scent-marking, followed by the suprapubic scent-gland. This was also found in another study on cotton-top tamarins (French and Cleveland, 1984), as well as in other tamarins: red-bellied tamarins (Coates and Poole, 1983), moustached tamarins, Geoffroy's saddleback tamarins, pied tamarins, *S. bicolor*, and golden-mantled tamarins, *S. tripartitus*

(reviewed in Heymann, 2001). Conversely, the silvery marmoset group at TZ scent-marked more frequently using their suprapubic scent-gland. This was also found in Aripuaña marmosets, and Santarem marmosets, *Mico humeralifer* (formerly genus *Callithrix*; Rylands, 1984, 1990). However, Omedes (1981) recorded only anogenital and sternal scent-marks in captive silvery marmosets, and Lazaro-Perea, Snowdon and Arruda (1999) showed that wild common marmosets produced primarily anogenital scent-marks. In addition, some authors have considered a more complex way to distinguish scent-marking types; for instance Rylands (1990) made the distinction between single suprapubic or sternal marks, and suprapubic mark directly followed by sternal mark. Histologically, the three callitrichid scent-glands have very similar aspects (Fontani et al., 2014; Perkins, 1966, 1975). Nonetheless, the chemicals secreted by each gland might vary in diversity and/or concentration, potentially leading to the production of different scent signals. A handful of studies have identified differences in the chemical composition of various scent-glands in other mammalian species, notably between genital and brachial scent-gland secretions in the ring-tailed lemur (Scordato, Dubay and Drea, 2007), subcaudal and pectoral scent-gland secretions in the owl monkey (Spence-Aizenberg et al., 2018), and cloacal and spur wax secretions in the echidna, *Tachyglossus aculeatus* (Harris et al., 2014). These studies suggest that different signals may be conveyed by each of these glands (Greene et al., 2016a). I will further assess this hypothesis in **Chapter 4**, by comparing the chemical composition of anogenital, suprapubic, and sternal scent-glands of two wild sympatric tamarins.

In addition, the duration of scent-mark deposition recorded in the present study varied. While all groups performed both short (i.e. 1–2 sec), medium (i.e. 3–6 sec), and long (i.e. >6 sec) scent-mark depositions, silvery marmosets at TZ preferentially performed short depositions, and emperor tamarins and cotton-top tamarins at DMP produced mainly scent-marks of medium duration. This variation in scent-marking duration might be explained by differences in body mass in these three species. On average, silvery marmosets have a lower body mass (♂ 0.330–♀ 0.360 kg) than emperor tamarins (♂ 0.474–♀ 0.475 kg) and cotton-top tamarins (♂ 0.418–♀ 0.404 kg; Smith and Jungers, 1997); hence they may produce less glandular secretions, which are quicker to deposit, than the two tamarin species. Alternatively, as we can assume that the amount of secretion

deposited on the substrate is proportional to the duration of the scent-marking event, the differences observed may reflect different scent-marking strategies, leading to the transmission of different signals (Müller-Schwarze, 2006). Additionally, if scent-marking is a visual signal as well as an olfactory one, the use of various fluids, scent-glands, and duration of deposition, may constitute a variety of immediate visual signals able to be conveyed to the receivers (Johnstone, 1996; Kappeler, 1998; Palagi and Norscia, 2009).

II.4.1.3. Temporal and spatial characteristics of scent-marking activity

There was no global variation of scent-marking frequency between days of observation, which was expected given the short-term design of the study (i.e. 10 days). Had the study been longer, we may have expected variation in scent-marking behaviour across seasons, as well as with changes in the groups' social dynamic (e.g. reproductive state of the dominant female, subordinate individuals escaping reproductive suppression, removal of an individual, birth of new offspring, etc.; Wyatt, 2014a). Scent-marking behaviour of the two emperor tamarin groups varied with the time of day: they scent-marked more frequently in the afternoon than in the morning and around midday. A similar result was found by Nogueira et al. (2001) on captive groups of common marmosets; however this was in opposition to a field study by Bartecki and Heymann (1990), which found that Geoffroy's saddleback tamarins scent-marked nearly twice as much in the morning than in the afternoon. Wild emperor tamarins in south-eastern Peru also tend to scent-mark more frequently in the morning (Watsa, pers. comm.), which is a highly active foraging and travelling period in their daily routine. The difference observed between these field studies and the present captive study likely arises from the constraints of captive environments, in which food availability and light intensity are artificially set. Alternatively, restrictions of observations in the present study may have omitted a highly active phase of scent-marking activity, if this happened before the beginning of daily observations (i.e. ca. 09:00 h). Indeed, a captive study of common marmosets showed a bimodal pattern of scent-marking behaviour, where the animals marked the most in the 06:00–07:00 h, and 15:00–16:00 h intervals (Sousa, Nogueira Moura and de Lara Menezes, 2006). Scent-marking frequency appeared constant throughout

the day for the silvery marmosets at TZ and the cotton-top tamarins at PWP, which was also observed by Smith (1997) in wild moustached and Geoffroy's saddleback tamarins.

All callitrichid groups studied were provided with three or more enclosure areas, separated by walls or mesh covered with branches and platforms. Silvery marmosets at TZ and cotton-top tamarins at DMP scent-marked almost exclusively in their indoor enclosure areas, while the group of emperor tamarins at TZ scent-marked both indoors and outdoors; which matched the general use of space for these groups. The difference observed between these groups is probably owing to the fact that the emperor tamarin group at TZ was observed in the spring, while the silvery marmoset group at TZ and both tamarin groups at DMP were studied in the winter, when the temperature and daylight length had diminished and the animals rarely went outdoors. Nevertheless, the cotton-top tamarin group studied at PWP very rarely scent-marked outdoors despite the fact that it was observed during summer, and the emperor tamarin pair at DMP never marked outdoors, yet both groups spent over 8% of their time outdoors in general. These groups appeared to have a preference for scent-marking in the indoor enclosure areas compared with the outdoor one. However, this result may have been an artefact of the difficulty of observations of these groups, for which visual access to the outdoor enclosure required the observer to leave the building housing the indoor enclosure and walk around it before the animals could be in sight again. These conditions made it more challenging to keep sight of the animals at all times compared with the other groups, and it is quite possible that some scent-marks were missed, especially at times when some animals were moving rapidly between the indoor and outdoor areas.

The enclosures of all callitrichid groups studied were provided with various branches, ropes and platforms, all constituting potential scent-marking substrates. The two main substrates chosen to scent-mark were horizontal and inclined branches. This result is similar to those found by Smith (1997) and Heymann (2001) on wild moustached and Geoffroy's saddleback tamarins. This preference may be explained by the fact that it is easier for the animal to rub its scent-gland on a horizontal or inclined substrate, than it is on a vertical substrate, although other species, such as the ring-tailed lemur (Palagi, Dapporto and Borgognini Tarli, 2005), and the

giant panda, *Ailuropoda melanoleuca* (White, Swaisgood and Zhang, 2003; Swaisgood, Lindburg and Zhou, 1999), are known to perform 'hand-stand scent-marking' where they stand on their hands and rub their anogenital area on a vertical tree trunk. In addition, as in Heymann (2001), the use of inclined branches to scent-mark was variable, with silvery marmosets at TZ and cotton-top tamarins at DMP using inclined substrates as much as horizontal ones, while the other groups favoured horizontal substrates. Nevertheless, the choice of substrate to deposit scent-marks may be correlated with the general time spent on each substrate, such as was found for the choice of enclosure area. It may also depend on the location of the substrate with respect to key elements of the enclosure, such as the sleeping box, feeding platforms, or door entrances, and the placement of the potential receivers. Unfortunately, the data collected for this study only included information on substrate inclination, which was insufficient to test further the hypothesis of a role of scent-marking in food resource location, territoriality, and/or orientation.

II.4.1.4. Influence of the presence and identity of conspecifics on scent-marking behaviour

Although in all callitrichid groups most individuals scent-marked in presence of one or several conspecifics as well as on their own, they did more frequently when in presence of several conspecifics, except in the case of the pair of emperor tamarins at DMP, which scent-marked as often on their own as they did when the other individual was present. This suggests that despite the fact that olfactory signals may linger in the environment until well after departure of the signallers, there may also be a choice to aim scent-marks at particular individual receivers. This was suggested in the case of acoustic communication, notably in Wield's black tufted-ear marmosets, *C. kuhlii* (Smith et al., 2009), pygmy marmosets (Snowdon and Elowson, 1999), squirrel monkeys, *Saimiri sciureus* (Biben, Symmes and Masataka, 1986), and Japanese macaques, *Macaca fuscata* (Arlet et al., 2015), in the context of social bonding; in Barbary macaques (Pfefferle et al., 2008a; b), in the context of sexual behaviour; and in chimpanzees, *Pan troglodytes* (Schel et al., 2013), in the context of food sharing. In addition, the fact that signallers often scent-marked in sight of conspecifics further supports the idea of scent-marking behaviour having a visual function as well as an olfactory one. Kappeler (1998), and Palagi and

Norscia (2009), demonstrated that ring-tailed lemurs perform ostentatious urine-marking with their stripy tail erected, not unlike tomcats do, while simple urination is done tail down. Furthermore, experimental bioassays by Kulahci et al. (2014) showed that ring-tailed lemurs can recognize individuals through olfactory–auditory matching. Such redundant signalling across several communication modalities may enhance signal transmission and maximize signal detection; it is widespread in the animal kingdom (Johnstone, 1996; Liebal et al., 2014; Moreira, Pessoa and Sousa, 2013).

Results from the present study at the individual level provided further indication that scent-marking behaviour might constitute a directional communication from signallers to receivers within the group, as some individuals scent-marked significantly more when next to certain conspecifics than others. For example, the male silvery marmoset at TZ scent-marked mostly next to his mate, which could indicate a role of the scent signals in reproduction, perhaps an incitement to mate; and next to the juvenile and subordinate females, which could serve as a dominance signal. Both the subordinate male and the subordinate female silvery marmoset at TZ scent-marked more when they were alone in their enclosure area, where they may have been avoiding dominants reacting to their marks. It could also be evidence of them trying to escape their subordinate status by looking for reproductive opportunities away from their parents, a strategy described in female callitrichids in the previous section (Lazaro-Perea, 2001; Sousa et al., 2005). Furthermore, the subordinate male emperor tamarin at TZ principally scent-marked next to the reproductive male, a potential sign of challenge, or on the contrary, of submission, towards his father. In the cotton-top tamarin group at PWP, the reproductive female marked more next to the reproductive male and the two subordinates, possibly informing on her reproductive state as well as dominance status. Finally, the reproductive female cotton-top tamarin at DMP scent-marked more next to the reproductive male, and one of the juvenile males, than to his twin; and this same juvenile scent-marked more when he was next to his mother. This may indicate an effort of the juvenile to imitate his mother's own scent-marking behaviour, as it is well established that young mammals learn a lot by imitation (Thorpe, 1956). Nevertheless, care must be taken when interpreting a signaller's 'choice' of neighbour when scent-marking, as it is not possible to distinguish a situation where the individual signaller decides to position itself next to a

particular conspecific for marking, from one where a conspecific moves close to the signaller individual just before scent-marking.

When looking at individual investigatory responses to scent-marks, in the form of sniffing, muzzle-rubbing, or overmarking, marks deposited by male and female callitrichids in this study were mainly investigated by individuals of the opposite sex, which further suggests a reproductive function of scent-marking behaviour. Yet overall, although most recorded scent-marks were produced in presence of given conspecifics, few of them seem to have elicited an investigatory behaviour. This result contrasts with other primate studies; for instance, Kappeler (1998) found that, in semi free-ranging ring-tailed lemurs, 62% of deposited scent-marks were investigated, with a median latency of 30 sec. Nonetheless, the chemical signals conveyed by scent-marks may trigger quick responses, called primer effects (Brown and Macdonald, 1985), at endocrinal and neurological levels, which would not be visible to the human observer (e.g. Laska et al., 2004; Laska, Wieser and Hernandez Salazar, 2005, in several species of primates; Snowdon et al., 2006, in callitrichids; Roberts and Gosling, 2004, in harvest mice, *Micromys minutus*; reviewed in Wyatt, 2014a). Several other studies of the primate order have reported important rates of investigatory response over scent-marks deposited by conspecifics. For instance, Epple, Kuderling and Belcher (1988) showed that scent-marks from novel female cotton-top tamarins elicited more investigatory responses from conspecifics than those from females to which the subjects had been habituated. Smith and Gordon (2002), as well as Kappeler (1998), observed that captive male red-bellied tamarins, and ring-tailed lemurs, respectively, investigated scent-marks from the dominant, reproductive female, more than scent-marks from other group members. Heymann (1998) found a similar result when examining investigatory responses to scent-marks in a wild population of sympatric moustached and Geoffroy's saddleback tamarins. In the case of the present study however, it is more likely that the way the data were collected did not correctly reflect the range of responses elicited by deposited scent-marks. Indeed, in this study responses to scent-marks were only recorded if they happened within 2 min of deposition. The reason for this was simply logistical: a single observer would not have been able to monitor older scent-mark spots for later responses, as well as recording *ad libitum* scent-marking activity and individual proximity measures every 2 min. This aspect

of data recording may have led to mislabelling overmarks for scent-marks, or sniffing in response to a mark for sniffing in the context of foraging.

II.4.2. Limitations of the study

The sample size in the present study was relatively small, thus limiting the interpretations from the results discussed in this chapter to hypotheses. In particular, the three species studied were observed in three different sites, which did not permit discrimination between interspecific and site differences in scent-marking behaviour. Moreover, only one group of silvery marmosets was observed, and the five callitrichid groups were of different sizes and composition. A more balanced study design would have included at least two groups of each species, composed of the same number of individuals, and sites housing all three species, to disentangle species and site differences. Unfortunately, study sites large enough to allow such a study could not be found in the UK, and access to farther sites in other parts of Europe and the world was beyond the scope of this PhD, both in terms of logistics, costs, and permissions. Nevertheless, as seen in the previous discussion sections, the present results were consistent with other existing studies on callitrichids and mammals in general, hence giving them value.

In addition, a possible drawback of the current study may have been to consider multiple scent-marks deposited by the same animal over a short time as distinct scent-marking events. Other studies, such as Heymann's on wild moustached and Geoffroy's saddleback tamarins (Bartecki and Heymann, 1990; Heymann, 2001), have considered a sequence of scent-mark deposition as the unit of scent-marking event. Heymann even quantified the intensity (i.e. the number of scent-marking acts per event) and complexity (i.e. the number of scent-marking types per event) of scent-marking behaviour. It is therefore possible that the present data comparing scent-marking type, duration, and behavioural response, did not reflect the reality. The choice of scent-marking recording was motivated by the constrained space in which the callitrichid groups were studied. Indeed, whilst in the wild scent-marking events may be well spaced out, and thus readily discernible, in captivity however it can be difficult to distinguish a sequence of scent-marks from independent scent-marking events.

Considering each scent-marking act as an independent event seemed a more conservative way to proceed.

II.4.3. Conclusion

The present study showed differences in scent-marking behaviour at the levels of species, group, sex, reproductive status, and the individual. Moreover, the presence and identity of conspecifics influenced an individual's scent-marking activity. In addition, scent-marking activity differed in type and duration, as well as scent-gland used. Finally, differences in enclosure area and substrate use were further observed. Interspecific, intersexual, and individual variation in scent-marking behaviour, including the frequency of marking, scent-gland use, duration of the marking, and choice of substrate, may reflect different strategies of communication to ensure that signals are conveyed to the intended receivers, which is especially relevant for species living sympatrically.

Chapter III – Scent-marking semiochemistry in captive callitrichids

Abstract

This chapter examines variation in the chemical composition of captive primate scent samples. First, I evidenced differences in chemical richness (i.e. the number of compounds present in a sample) and chemical diversity (i.e. the combination of individual compounds in a sample) between sample types (i.e. glandular secretions, deposited scent-marks and urine), species, tamarin groups, and at the levels of sex, reproductive status, and individual females. Moreover, I verified the identity of a subset of 47 compounds retrieved from the samples; and discussed their chemical aspects and prevalence in the different categories of sample tested. In addition, I experimentally tested the temporal stability of sample chemical composition. Results from this study support the idea of a role of chemosignalling in callitrichids in species and group recognition, as well as in mate choice and intrasexual competition. Furthermore, this study reveals some aspects of the complex chemical composition and temporal stability of callitrichid scent signals, and enhances our current knowledge of putative mammalian semiochemicals.

III.1. Introduction and hypotheses

III.1.1. Callitrichid semiochemicals

In callitrichids, secretions produced by three specialized scent-glands on the sternal, suprapubic, and anogenital areas of the body, are deposited on branches in the environment through conspicuous scent-marking behaviours (Epple et al., 1993). Con- and hetero-specifics often inspect these scent-marks by sniffing, licking, or muzzle-rubbing the marked spots (Smith et al., 1997). They also perform this investigative response to voided urine, suggesting that both scent-marks and urine play

a role in chemosignalling in this taxon. The combination and relative concentration of volatile compounds picked up from a deposited scent-mark or urine pool constitute a potentially unique chemical message (Wyatt, 2014a).

As early as 1981, Epple suggested that there might be species differences in callitrichid scent-gland secretion chemical composition (Epple et al., 1981; Epple, Kuderling and Belcher, 1988). Several more recent publications on the Primate order suggest that chemical signatures at the levels of species, group, sex, reproductive status, and the individual, can be conveyed in the chemical profiles of scent-gland secretions (e.g. in Coquerel's sifakas, *Propithecus coquereli*, Greene and Drea, 2014; common marmosets, *Callithrix jacchus*, Smith et al., 2001b; and owl monkeys, *Aotus* spp., Spence-Aizenberg et al., 2018), deposited scent-marks (e.g. in ring-tailed lemurs, *Lemur catta*, Scordato, Dubay and Drea, 2007; and mandrills, *Mandrillus sphinx*, Setchell et al., 2010; Vaglio et al., 2016), and urine (e.g. in brown lemurs, *Eulemur* spp., DelBarco-Trillo and Drea, 2014).

As previously mentioned in **Chapter 1**, relatively few studies have investigated the identity of primate semiochemicals, in comparison with other mammalian taxa such as carnivores and rodents (Heymann, 2006b). The main classes of semiochemicals found in studies of Lemuridae (Boulet et al. 2009; Hayes et al. 2004; Knapp et al. 2006; Scordato et al. 2007), Callitrichidae (Belcher et al., 1988; Epple et al., 1981; Smith et al., 2001b), Aotidae (MacDonald et al., 2008), Cercopithecidae (Birkemeyer et al. 2016; Setchell et al. 2010), and Hominidae (De Lacy Costello et al., 2014; Matsumoto-Oda et al. 2003), were short- and long-chained carboxylic acids and their esters, ketones, and aldehydes, as well as hydrocarbons, fatty alcohols, aldehydes, alkenes, terpenes, and sterols.

III.1.2. Aims and hypotheses

This chapter explores variation in the chemical composition of captive callitrichid scent samples, and tests for chemical signatures at the levels of sample type, study site, species, group, sex, reproductive status, and the individual. Differences between sample categories can be assessed by comparing their chemical richness, i.e. the number of

compounds present in the sample, as well as their chemical diversity, i.e. the combination of individual compounds in a sample and their commonness among samples.

First, I tested whether the chemical composition (i.e. chemical richness and diversity) of various types of sample, i.e. scent-marks, scent-gland secretion and urine, differed, and differed from that of branch and air samples collected inside the enclosures. Voided urine and scent-gland secretions originate from two different biochemical pathways; therefore, they can be expected to contain a very different range of compounds. Moreover, chemical changes are likely to happen after the deposition of scent-gland secretions, owing to bacterial activity once in the open air, and reactions with existing compounds on the substrate (Ezenwa and Williams, 2014; Theis et al., 2013). Consequently, scent-mark swabs may be of different, potentially more complex, chemical composition than scent-gland secretion swabs collected directly from the animals. I then examined differences between study sites, species, and groups. The callitrichid groups housed at each of the three zoos included in the present study have different genetic backgrounds, which is likely to translate into chemical differences. For instance, Kean et al. (2017) presented evidence for 'odour dialects' in genetically distinct subpopulations of Eurasian otter, *Lutra lutra*, across the UK. Besides, differences in husbandry procedures (e.g. diet, cleaning, and enrichment routines) at the three study sites might be reflected in the chemical composition of callitrichid scents. Indeed, Drea et al. (2013) found chemical differences between scent-marks of ring-tailed lemurs fed different diets. In addition, I investigated the existence of chemical signatures at the levels of sex, reproductive status, and the individual. As suggested in **Chapter 2**, these may play a role in mate choice, intrasexual competition and reproductive suppression in callitrichids (Heymann, 2006a; Snowdon et al., 2006).

Second, I inspected the putative identity of the compounds retrieved from the samples, verified this identity for a subset of compounds of interest, and described the main chemical aspects of these putative semiochemicals. I then compared the prevalence of individual compounds across the different categories of samples tested (i.e. sample type, study site, species, group, sex, reproductive status, and individual females). We

can hypothesise that different compounds may code for chemical signatures at different levels.

Finally, I experimentally assessed the temporal stability of sample chemical composition. The existing literature on mammalian semiochemistry unanimously recommends researchers in this field to store and transport scent samples frozen, because the high volatility of their chemical components makes them prone to degrade very quickly at room temperatures (e.g. Charpentier et al., 2012; Drea et al., 2013). However, the exact pattern of degradation for this category of samples, i.e. a gaseous headspace above the swabs, is not known. Here I experimentally tested patterns of sample decay, by extracting samples multiple times and controlling for the delay between extractions. I compared different conditions of time delay at room temperature, and different numbers of successive extractions. The conditions tested aimed to ***i.*** confirm that samples degraded over time when not kept frozen, ***ii.*** test whether the time spent at room temperature, or the number of extractions, had the greatest effect on sample degradation or change, and ***iii.*** describe the pattern of degradation for this category of samples.

III.2. Methods

III.2.1. Odorant sample collection

Scent-mark and urine samples were collected from two groups of bearded emperor tamarins, *Saguinus imperator subgrisescens* (later referred to as *S. imperator*) housed at Drayton Manor Park (DMP) and Twycross Zoo (TZ), along with two groups of cotton-top tamarins, *S. oedipus*, housed at DMP and Paradise Wildlife Park (PWP; **Table III.1**). All three study sites are members of the British and Irish Association of Zoos and Aquariums (BIAZA). This project was approved by the Anglia Ruskin University, Faculty of Science and Engineering, Departmental Research Ethics Panel committee (DREP), and BIAZA.

Scent-marks and voided urine were located during continuous observation of the tamarin groups from the public area of the zoo (50 hours of observation per group, see **Chapter 2**). Scent-marking events were systematically recorded for the purpose of the behavioural study detailed in

Chapter 2. A subset of these recorded scent-marks was sampled for emperor and cotton-top tamarins in the few cases in which access inside the animals' enclosure was made possible shortly after deposition (i.e. less than 10 min). A number of samples were also collected outside the behavioural observation time, depending on the availability of the members of staff at each zoo. Moreover, only scent-marks that had not been overmarked or stepped on by other tamarins after initial deposition were collected. In addition, veterinarians at TZ collected suprapubic scent-gland samples from one group of emperor tamarins and one group of another callitrichid species, the silvery marmoset, *Mico argentatus*, during routine health checks. Scent-mark and urine samples were also collected opportunistically from two other New World primate species, the white-faced saki monkey, *Pithecia pithecia*, and the black-headed spider monkey, *Ateles fusciceps*, at DMP.

Collection of scent-marks and urine was performed by swabbing the branch spot (usually a wet mark was visible to help locate the secretion), using a clean 1–2 cm^2 square of viscose gauze – thereafter referred to as swab – held by clean forceps. Forceps were wiped with methanol prior to each swab collection. Scent-gland secretion collection was similarly achieved by gently rubbing a swab over the scent-gland area a few times (**Fig. III.1a**). Swabs were kept individually in 4 mL glass chromatography vials closed by a screw-top polytetrafluoroethylene septum lid (**Fig. III.1b**).

Prior to use, both vials and swabs were washed in HPLC-grade methanol and pentane (ACROS Organics™, London, UK), then baked at 130°C for 30 min prior to use, as recommended by Birkemeyer et al. (2016). After collecting the secretion, the swab was quickly returned to its vial and closed, and the forceps were wiped on clean gauze with pentane. Sample vials were kept in an insulated cool box filled with frozen gel packs at a temperature close to 0°C, then stored in a freezer onsite (-15°C at DMP and PWP, -20°C at TZ) within two hours (usually 30 min). Swabs from the enclosure branches where no scent-mark was deposited (branch swabs), and swabs exposed for 30 sec to the ambient air inside the enclosure (air swabs), were also collected. At the end of each data collection period, the samples were transported in the cool box to Anglia Ruskin University (ARU), where they were stored at -80°C until analysis.

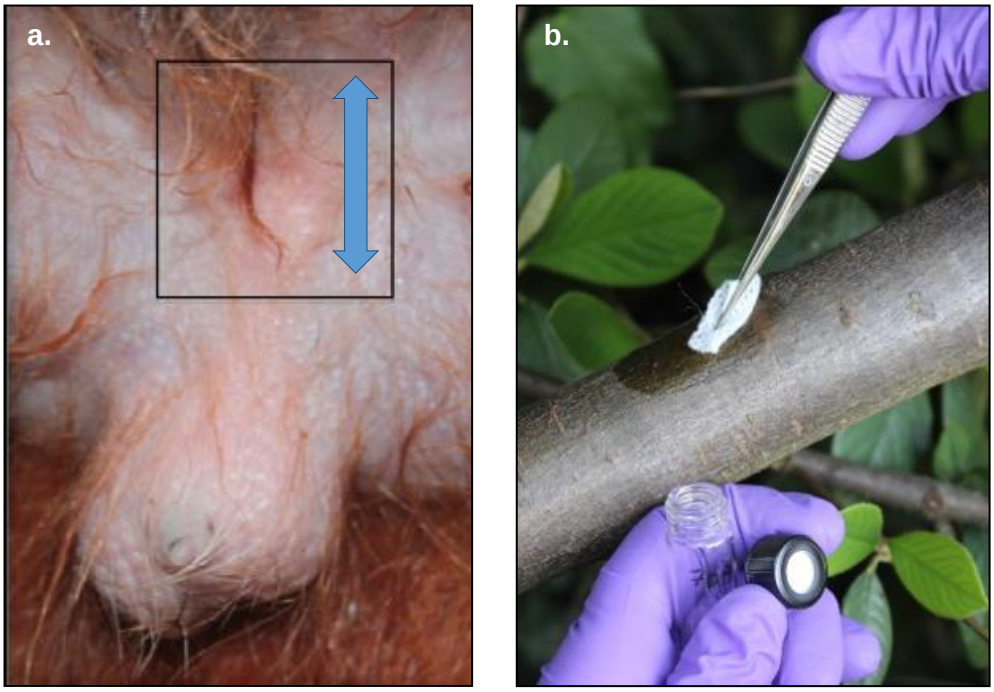


Figure III.1. a. Genital area of an adult male emperor tamarin, *Saguinus imperator* (from Watsa, 2013). The suprapubic gland (indicated by a box) is a pink coloured, granulated skin surface. Sampling procedure consists of gently wiping a gauze swab, several times up and down (indicated by the blue arrow); **b.** Naturally deposited scent-mark sample collection, using a gauze swab rubbed on the scent-mark spot, and stored in a glass vial.

III.2.2. Sample extraction and GC-MS analyses

Samples were analysed one by one using headspace solid-phase microextraction (SPME) and gas chromatography-mass spectrometry (GC-MS) at ARU. Each sample was retrieved from the -80°C freezer just before analysis, and placed in a heat block at 40°C for an equilibration period of 10 min. Samples were extracted using a 65 µm polydimethylsiloxane/ divinylbenzene StableFlex™ SPME fibre (Supelco, Bellefonte, PA, USA) for a period of 30 min at 40°C. The sample-coated fibre was then manually injected at 250°C into the injection port of a Clarus 500 GC (PerkinElmer), fitted with a Thermogreen® LB-2 pre-drilled septum, and a splitless 1 mm liner. A flow of helium of 1 mL/min was used as carrier gas. Splitless mode was applied for injection, which meant that all the material desorbed from the fibre was allowed to enter the column at the time of injection. A non-polar, thermally stable capillary column, made of polydimethylsiloxane cross-linked with 5% divinylbenzene (30 m x 0.25 mm x 0.25 µm film

thickness, Equity™ 5, Supelco) was used. The oven temperature programme started at 40°C, held for 2 min, followed by an increase of 6°C/min to the final temperature of 200°C, held for 8 min. A cool-down ramp was added, decreasing the temperature to 40°C at 20°C/min, held for 4 min. The total run lasted 43 min. The electron ionization Clarus 500 MS (PerkinElmer) was equipped with a quadrupole, and set to scan for mass-to-charge ratios between 41–300 m/z, from 2–40 min. These scanning parameters were set after a refining process aimed to reduce baseline noise to a minimum. Before each sample was analysed, the fibre was conditioned 1 min at 250°C in the injection port of the GC-MS; then a blank run, in which nothing was injected, was performed, to ensure the GC column was clean. Samples from each of the three collection sites were analysed in random order to ensure that no batch effect was artificially created. However, the GC-MS instrument was serviced between the analysis of samples from TZ and PWP and that of samples from DMP, which may have led to a slight variation in the analytical performance.

The resulting chromatograms were converted from .raw to .cdf format using the open access software OpenChrom® (Wenig and Odermatt, 2010), and further analyses were carried out using the software ChemStation™ (Agilent, Santa Clara, CA, USA). Blank fibres, empty vials and vials containing an unused swab were added to the pool of samples to analyse, thereafter referred to as blank samples, in order to identify extraneous contaminant compounds in the samples. Over 30 samples were collected from each site, some of which were repeat samples from the same animals. Sample quality was evaluated on the basis of sample freshness (i.e. the delay between scent-marks or urine deposition, and collection), cool box temperature, latency to storage in a freezer, cleanliness of the GC column at time of analysis (assessed via a blank run prior to analysing the sample), SPME fibre ageing (i.e. the number of times the fibre had been used prior to the analysis), and any unforeseen event during sample analysis. A total of 95 best-quality samples, including animal, branch and air samples, were ultimately included in the present analysis (**Table III.1**).

Table III.1. Number of good quality animal samples (i.e. scent-marks, SM, scent-gland secretions, SG, and urine, Ur), as well as air and branch (Br) controls, collected from each individual composing the seven primate groups included in the study.

Species studied	**Study Site**	**Group composition**	**Number of samples**					**Total per group**
			SM	SG	Ur	Air	Br	
Emperor tamarin, *S. imperator*	DMP	Repro. ♂	1	0	1	2	2	13
		Repro. ♀	5	0	2			
	TZ	Repro. ♂	1	0	1	3	4	40
		Repro. ♀	9	1	5			
		Subord. ♂	4	1	0			
		Subord. ♀	4	1	1			
		Juvenile ♂	1	1	1			
		Juvenile ♀	1	1	0			
Cotton-top tamarin, *S. oedipus*	DMP	Repro. ♂	0	0	0	2	2	8
		Repro. ♀	2	0	1			
		Juvenile ♂1	0	0	1			
		Juvenile ♂2	0	0	0			
	PWP	Repro. ♂	2	0	1	3	5	23
		Repro. ♀	3	0	1			
		Subord. ♂	1	0	1			
		Subord. ♀	4	0	0			
		Juvenile ♂	1	0	0			
		Juvenile ♀	0	0	1			
Silvery marmoset, *M. argentatus*	TZ	Repro. ♂	0	1	0	0	0	6
		Repro. ♀	0	0	0			
		Older subord. ♀	0	0	0			
		Subord. ♂	0	1	0			
		Subord. ♀	0	1	0			
		Juvenile ♂	0	1	0			
		Juvenile ♀	0	1	0			
		Infant	0	1	0			
White-faced saki monkey, *P. pithecia*	DMP	Adult ♂	1	0	0	1	1	3
		Adult ♂	0	0	0			
Black-headed spider monkey, *A. fusciceps*	DMP	Subadult ♀	0	0	2	0	0	2
		Unk.	0	0	0			
Total samples	All	All	**40**	**11**	**19**	**11**	**14**	**95**

In addition, a subset of lower-quality scent-mark and urine samples was used to experimentally investigate the pattern of sample decay. Three conditions were tested, under which samples were analysed by SPME–GC-MS between two and five times and kept at room temperature (ca. 20°C) between each run. Under the first condition, samples were analysed five times at short interval, at time 0 h, 1.5 h, 3 h, 4.5 h, and 5 h. As the analysis of each sample lasted 1.5 h, including equilibration, extraction and GC-MS run, this was the shortest time interval applicable. Under the second condition, samples were analysed five times at long time interval, at time 0 h, 10 h, 24 h, 48 h, and 72 h. Under the third condition, samples were analysed only twice at the maximum time interval, i.e. at time 0 h and 72 h. Six repeats of each condition were run.

III.2.3. Interpretation of analytical results

For each GC-MS chromatogram, automatic peak detection, integration, and tentative identification using the National Institute of Standards and Technology (NIST) mass spectral library (Shen et al., 2014), was performed in ChemStation™. Only peaks with a minimum height of 1% of that of the largest peak were selected, in order to limit the inclusion of background noise. All detected peaks were listed using the information of retention time, peak area and height, mass spectrum, and putative NIST identification. Careful visual comparison of the peaks' mass spectra made it possible to determine whether peaks of similar retention times represented the same or different compounds. In addition, all peaks found in at least one of the blank samples were classified as contaminants and removed from the list of compounds of interest. These time-consuming steps were essential, as the results of the NIST library cannot be taken for a certainty, especially when the compounds identity matches given by the library were below 80%. The identities of 22 compounds were further confirmed by comparison of their retention times with those of commercially obtained compounds, analysed under identical conditions. However, as the main objective of this study was to investigate differences in chemical composition between samples, rather than to name individual compounds, patterns can be discussed without knowing the definite chemical identity of each compound (e.g. Charpentier, Boulet and Drea, 2008; Kean, Chadwick and Müller, 2015).

III.2.4. Statistical analyses

All statistical analyses were performed in R v.3.5.1 operated in RStudio (R Core Team, 2018). Chemical richness (i.e. the total number of compounds retrieved from a sample), and chemical diversity (i.e. the combination of compounds in a sample) were examined across categories of samples: sample types, study sites, species, groups, sexes, reproductive status, and individuals. While sample type was compared between all classes of samples, the other categories were tested only on a subset of the data, owing to the variable sample size across categories. For this reason, four distinct nested datasets were created (**Table III.2**), which are referred to in the text and figures throughout the analyses in order to avoid confusion.

Table III.2. Nested datasets used to visualize and test the significance of differences in samples chemical richness and chemical diversity between sample types, study sites, species, and tamarin groups, sex, reproductive status, and individual.

#	Dataset name	Samples included in dataset	Number of samples
1.	All samples dataset	All samples	95
2.	Animal samples dataset	Only animal samples, no branch and air samples	70
3.	Tamarin samples dataset	Only emperor and cotton-top tamarin scent-mark and urine samples, no branch and air samples	56
4.	Adult female tamarin samples dataset	Only samples from reproductive and non-reproductive adult females of the four groups of tamarins (i.e. six females)	37

Chemical richness was compared between sample types (all samples dataset), study sites and species (animal samples dataset), as well as groups (tamarin samples dataset), using non-parametric Kruskal-Wallis rank sum tests of difference (function kruskal.test() in R base package ‘stats’). In addition, Dunn’s tests with Bonferroni adjustment (i.e. pairwise tests for multiple comparisons of mean rank sums; function posthoc.kruskal.dunn.test() in R package ‘PMCMR’; Pohlert, 2014) were

used as post-hoc tests to assess pairwise differences within the same categories. Then, Kruskal-Wallis rank sum tests and post-hoc Dunn's tests were performed to investigate differences in chemical richness at the levels of sex and reproductive status (tamarin samples dataset), as well as the individual (adult female tamarin samples dataset), for each tamarin group independently. Additionally, a generalized linear mixed model (glmer() in R package 'lme4'; Bates et al., 2015) with *Poisson* family and log link function was fitted to assess the relative effect of species, sex, reproductive status, sample type, and their interactions, on the number of compounds retrieved in the tamarin samples dataset. Group, and individual nested into group, were included as random effects. Study site was not included in the models, as it was redundant with species and group. Determination of the variance inflation factor (vif() in R package 'car'; Fox and Weisberg, 2011) revealed no collinearity issue (max VIF= 3.6). Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality. Research of the best-fit model through model selection was not carried out since both significant and non-significant effects were discussed here.

A more detailed comparison of the chemical composition of the different types of samples was further conducted by assessing differences in chemical diversity. A classic way to measure chemical diversity is to compute diversity indices such as those used in ecology (Legendre and Legendre, 1998). For example, Charpentier, Boulet and Drea (2008) used the richness, Shannon and Simpson indices to assess seasonal variations in male ring-tailed lemurs' scent-gland chemical diversity. However, the chemical data in this study consisted of patterns of presence/absence of certain compounds in the different samples, but did not include a measure of compound abundance, thus preventing the use of these classic indices. Instead, the approach chosen was the use of Non-Metric Multidimensional Scaling (NMDS), which allowed quantification and graphical visualization of chemical diversity between groups of samples, followed by Permutational Multivariate Analysis of Variance Using Distance Matrices (PerMANOVA). Such analyses can be computed using the R package 'vegan' (Oksanen et al., 2017). This package was created for the multivariate analysis of ecological communities, offering ordination and diversity analysis methods to explore patterns of presence/absence, or abundance, of animal and vegetal species within an ecological community. It seemed well adapted to

the present study, and has notably been employed in semiochemical studies of Eurasian otters (Kean, Chadwick and Müller, 2015; Kean et al., 2017), and meerkats, *Suricata suricatta* (Leclaire, Nielsen and Drea, 2014; Leclaire et al., 2017).

The first step in assessing sample chemical diversity was to compute a distance matrix of samples and their respective compound composition (vegdist() R function), using the Jaccard dissimilarity index. This index is calculated as $J = 2B/(1 + B)$, where B is the Bray-Curtis dissimilarity measure between every pair of samples based on the relative compound composition (both in terms of richness and diversity). Second, three-dimensional NMDS coordinates were calculated from the values of Jaccard dissimilarity index in the distance matrix, with metaMDS(). The default number of dimensions for metaMDS() is two; however, using three dimensions reflected better the data ordination here, as measured by the stress factor. These three coordinates, plotted as scatterplots on the x–y, y–z, and z–x planes, allowed for the visualization of dissimilarity between groups of samples. Scores of three-dimensional stress factor measured the goodness-of-fit between predicted and observed values (similar to the R^2 value in a regression), considered a good fit when stress ≤0.2. Third, a PerMANOVA was carried out with adonis(), to test whether the observed differences in chemical composition between groups of samples were significant. The PerMANOVA test is a non-parametric method fitting a linear model to the distance matrix. It applies a permutation test to calculate the sequential sum of squares, mean squares, pseudo-*F* statistic, partial R^2, and *P*-value for each term in the model. In this analysis, 999 permutations and the Jaccard dissimilarity index were used. Unlike most statistical models, the only statistical assumption for adonis() is to ensure multivariate homogeneity of variance within each group tested. This assumption was tested using the permutation test for homogeneity of multivariate dispersion (permutest(), using 99 permutations), on the measure of group multivariate homogeneity of variance computed using betadisper(), prior to running adonis(). In addition, pairwise PerMANOVA tests (pairwise.adonis(); Martinez Arbizu, 2019) allowed for detailed comparisons of chemical diversity between pairs of sample categories. A multifactorial PerMANOVA (adonis() R function) was then built to assess the relative effect of sample type, species, group, sex, reproductive status, and the individual, on sample chemical diversity. This global model accounted for multicollinearity

between factors, where sex, reproductive status and individual were included as terms nested into group, and study site, which was redundant with species and group, was not included.

To investigate the temporal stability of scent samples under experimental conditions, Kruskal-Wallis rank sum tests were performed, followed by pairwise Dunn's post-hoc tests with Bonferroni adjustment. These tests compared the chemical richness of samples subject to different conditions, comprising the number of repetitive extractions and the delay between extractions. Sample stability was not tested for chemical diversity. Then, a generalized linear mixed model with *Poisson* family and log link function was built to further assess the effect of experimental conditions on sample chemical richness. The fixed effects in the model were experimental condition (i.e. delay between extractions) and extraction number; and the random effect was sample ID. Determination of the variance inflation factor revealed no collinearity issue (max VIF= 1). However collinearity appeared when adding interaction terms between experimental condition and extraction number; for this reason interactions were removed from the linear model. Visual inspection of residual plots did not reveal any obvious deviations from homoscedasticity or normality.

III.3. Results

III.3.1. Total number of compounds retrieved

A total of 407 different volatile compounds were revealed by SPME–GC-MS analysis of the 95 scent-gland secretion, scent-mark, urine, air, and branch samples included in this analysis (**Table III.1**; see **Appendix C**, **Table C.1**). Another 119 compounds were detected, which were classified as contaminants and removed from the pool of compounds, after comparison with blank samples (e.g. empty vials and empty fibres) and visual inspection of the mass spectra. Numbers of compounds in each sample varied from 3–94 (median= 18 ±SD 15.1 compounds). While 36.1% of compounds appeared to be unique to a sample, the rest were common to at least two samples (**Fig. III.2**). Only 1.2% of the compounds were present in more than half of the samples.

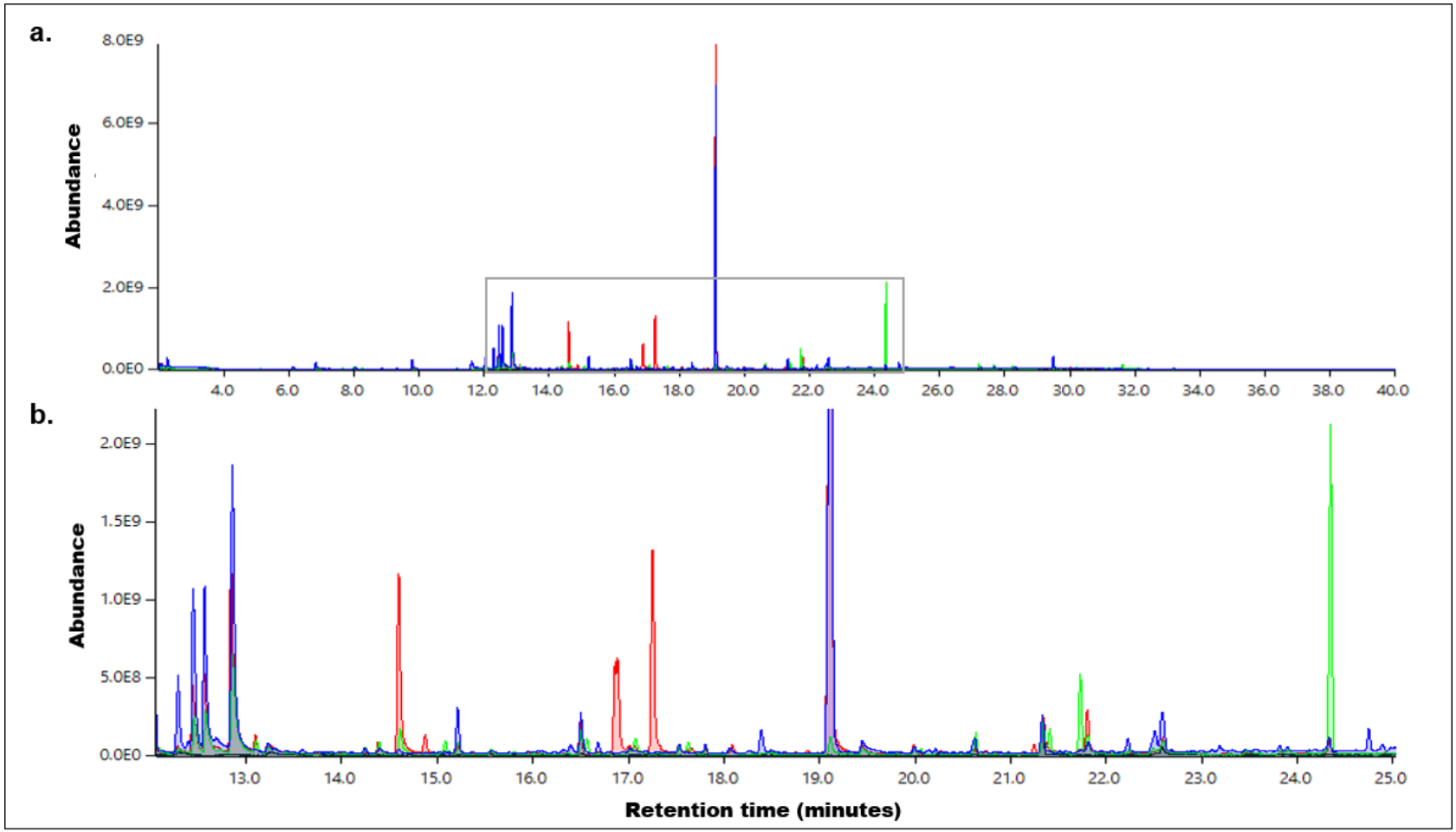


Figure III.2. a. Overlaid chemical profiles, and **b.** zoom of the section framed in grey, of scent samples obtained by SPME–GC-MS. Different colours indicate scent-mark samples from reproductive females emperor (in red) and cotton-top tamarin (in green) at DMP, and emperor tamarin at TZ (in blue), as well as a blank run (i.e. empty SPME fibre, in black). Each peak represents a unique compound; while some peaks are common to all three tamarin samples, i.e. overlapping peaks (e.g. at 14.5 and 19.1 min), others appear unique to a sample (e.g. at 17.2 and 18.4 min).

III.3.2. Variation in the chemical composition of scent samples

III.3.2.1. Variation in sample chemical richness

III.3.2.1.1. Differences between sample types

Chemical richness (i.e. the total number of compounds retrieved from the samples) differed significantly between sample types (**Table III.3**; all samples dataset). In particular, scent-mark, scent-gland and urine samples contained more compounds than air and branch samples (only significant for scent-mark–branch), whereas no difference was found between scent-mark, scent-gland and urine samples, nor between air and branch samples (**Fig. III.3a**; **Table III.3**). However, when comparing only samples from the two tamarin species, for which most samples were collected (tamarin samples dataset), chemical richness of scent-mark samples was significantly greater than that of urine samples (**Fig. III.3b**; **Table III.3**).

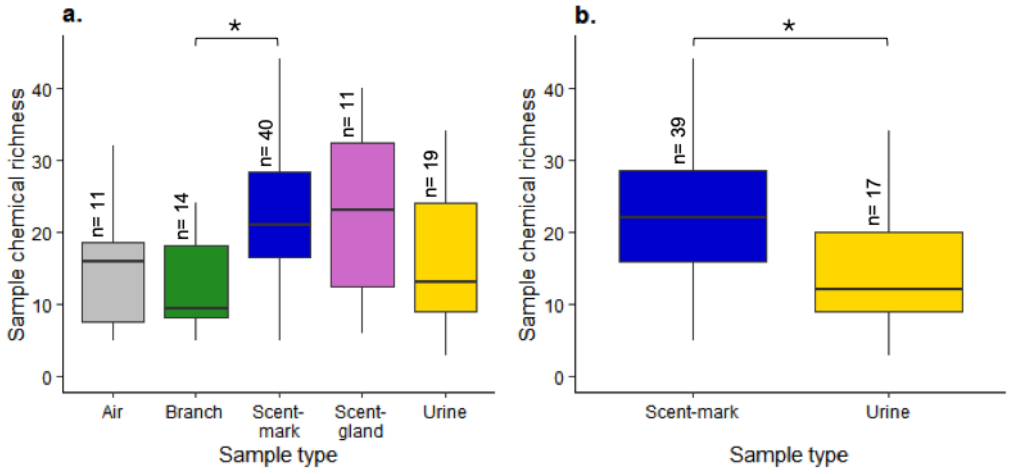


Figure III.3. Tukey's box-and-whiskers plots showing differences in sample chemical richness between **a.** all five sample types (all samples dataset); and **b.** scent-mark and urine of emperor and cotton-top tamarins (tamarin samples dataset). Boxes indicate the median and interquartile range (IQR); whiskers give the smallest value ≥ lower hinge -1.5*IQR and largest value ≤ upper hinge +1.5*IQR. n= sample size. Asterisks indicate statistically significant differences in pairwise Dunn's post-hoc tests (✶ $P \leq 0.05$, ✶✶ $P \leq 0.01$, ✶✶✶ $P \leq 0.001$; **Table III.3**).

Table III.3. Results of Kruskal-Wallis sum rank tests of difference, and Dunn's post-hoc tests on pairwise comparisons (for categories of sample size n >2), on sample chemical richness (i.e. number of compounds retrieved from the samples), for each category tested: sample type, study site, species, group, sex, reproductive status, and the individual. χ^2= χ^2-statistic; *df*= degrees of freedom; *Z*= *Z*-statistic; and *P*= *P*-value, significant at *P*≤ 0.05 (in bold).

Category tested	Dataset used	Kruskal-Wallis rank sum tests	Pairwise comparisons	Dunn's tests
Sample type	All samples dataset	χ^2= 11.416, *df*= 4, ***P*= 0.022**	Air – Branch	*Z*= 0.526, *P*= 1.000
			Air – Scent-gland	*Z*= 1.652, *P*= 0.980
			Air – Scent-mark	*Z*= 1.936, *P*= 0.530
			Air – Urine	*Z*= 0.508, *P*= 1.000
			Branch – Scent-gland	*Z*= 2.275, *P*= 0.230
			Branch – Scent-mark	*Z*= 2.805, ***P*= 0.050**
			Branch – Urine	*Z*= 1.148, *P*= 1.000
			Scent-gland – Scent-mark	*Z*= 0.134, *P*= 1.000
			Scent-gland – Urine	*Z*= 1.352, *P*= 1.000
			Scent-mark – Urine	*Z*= 1.674, *P*= 0.940
	Animal samples dataset	χ^2= 3.175, *df*= 2, *P*= 0.204	Scent-gland – Scent-mark	*Z*= 0.290, *P*= 1.000
			Scent-gland – Urine	*Z*= 1.452, *P*= 0.440
			Scent-mark – Urine	*Z*= 1.620, *P*= 0.320
	Tamarin samples dataset	χ^2= 6.333, *df*= 1, ***P*= 0.012**	Scent-mark – Urine	*Z*= 2.516, ***P*= 0.012**

Table III.3. Continued (1/3).

Category tested	Dataset used	Kruskal-Wallis rank sum tests	Pairwise comparisons	Dunn's tests
Study site	Animal samples dataset	χ^2= 4.738, *df*= 2, *P*= 0.094	DMP – PWP	*Z*= 0.786, *P*= 1.000
			DMP – TZ	*Z*= 2.106, *P*= 0.110
			PWP – TZ	*Z*= 1.127, *P*= 1.780
Species	Animal samples dataset	n too small	Spider monkey – Saki monkey	n too small
			Spider monkey – Silvery marmoset	n too small
			Spider monkey – Emperor tamarin	n too small
			Spider monkey – Cotton-top tamarin	n too small
			Saki monkey – Silvery marmoset	n too small
			Saki monkey – Emperor tamarin	n too small
			Saki monkey – Cotton-top tamarin	n too small
			Silvery marmoset – Emperor tamarin	*Z*= 3.040, ***P*= 0.024**
			Silvery marmoset – Cotton-top tamarin	*Z*= 2.589, *P*= 0.096
			Emperor tamarin – Cotton-top tamarin	*Z*= 0.413, *P*= 1.000
Group (1/2)	Tamarin samples dataset	χ^2= 13.650, *df*= 3, ***P*= 0.003**	Emperor at DMP – Emperor at TZ	*Z*= 3.677, ***P*= 0.001**
			Emperor at DMP – Cotton-top at DMP	*Z*= 2.069, *P*= 0.231
			Emperor at DMP – Cotton-top at PWP	*Z*= 2.491, *P*= 0.077
			Emperor at TZ – Cotton-top at DMP	*Z*= 0.310, *P*= 1.000

Table III.3. Continued (2/3).

Category tested	Dataset used	Kruskal-Wallis rank sum tests	Pairwise comparisons	Dunn's tests
Group (2/2)	Tamarin samples dataset		Emperor at TZ – Cotton-top at PWP	Z= 1.122, P= 1.000
			Cotton-top at DMP – Cotton-top at PWP	Z= 0.344, P= 1.000
Sex	Tamarin samples dataset	χ^2= 0.003, df= 1, P= 0.957	Male – Female	Z= 0.054, P= 0.960
	Tamarin samples dataset, only Emperor at DMP	χ^2= 1.059, df= 1, P= 0.306	Male – Female	n too small
	Tamarin samples dataset, only Emperor at TZ	χ^2= 0.510, df= 1, P= 0.475	Male – Female	Z= 0.714, P= 0.480
	Tamarin samples dataset, only Cotton-top at DMP	χ^2= 0.000, df= 1, P= 1.000	Male – Female	n too small
	Tamarin samples dataset, only Cotton-top at PWP	χ^2=0 .908, df= 1, P= 0.341	Male – Female	Z= 0.953, P= 0.340
Reproductive status	Tamarin samples dataset	χ^2= 0.733, df= 1, P= 0.392	Reproductive – Non-reproductive	Z= 0.856, P= 0.390
	Tamarin samples dataset, only Emperor at DMP	No non-reproductive individual		
	Tamarin samples dataset, only Emperor at TZ	χ^2= 1.581, df= 1, P= 0.209	Reproductive – Non-reproductive	Z= 1.257, P= 0.210
	Tamarin samples dataset, only Cotton-top at DMP	χ^2= 0.000, df= 1, P= 1.000	Reproductive – Non-reproductive	n too small
	Tamarin samples dataset, only Cotton-top at PWP	χ^2= 1.235, df= 1, P= 0.266	Reproductive – Non-reproductive	Z= 1.111, P= 0.270

Table III.3. Continued (3/3).

Category tested	Dataset used	Kruskal-Wallis rank sum tests	Pairwise comparisons	Dunn's tests
Adult female identity	Adult female tamarin samples dataset	χ^2= 11.801, *df*= 5, *P*= 0.038	Repro. ♀ Emperor at DMP – Repro. ♀ Emperor at TZ	*Z*= 3.042, ***P*= 0.035**
			Repro. ♀ Emperor at DMP – Repro. ♀ Cotton-top at DMP	*Z*= 1.776, *P*= 1.000
			Repro. ♀ Emperor at DMP – Repro. ♀ Cotton-top at PWP	*Z*= 1.007, *P*= 1.000
			Repro. ♀ Emperor at DMP – Subord. ♀ Emperor at TZ	*Z*= 2.731, *P*= 0.095
			Repro. ♀ Emperor at DMP – Subord. ♀ Cotton-top at PWP	*Z*= 1.598, *P*= 1.000
			Repro. ♀ Emperor at TZ – Repro. ♀ Cotton-top at DMP	*Z*= 0.288, *P*= 1.000
			Repro. ♀ Emperor at TZ – Repro. ♀ Cotton-top at PWP	*Z*= 1.370, *P*= 1.000
			Repro. ♀ Emperor at TZ – Subord. ♀ Emperor at TZ	*Z*= 0.366, *P*= 1.000
			Repro. ♀ Emperor at TZ – Subord. ♀ Cotton-top at PWP	*Z*= 0.717, *P*= 1.000
			Repro. ♀ Cotton-top at DMP – Repro. ♀ Cotton-top at PWP	*Z*= 0.778, *P*= 1.000
			Repro. ♀ Cotton-top at DMP – Subord. ♀ Emperor at TZ	*Z*= 0.511, *P*= 1.000
			Repro. ♀ Cotton-top at DMP – Subord. ♀ Cotton-top at PWP	*Z*= 0.293, *P*= 1.000
			Repro. ♀ Cotton-top at PWP – Subord. ♀ Emperor at TZ	*Z*= 1.442, *P*= 1.000
			Repro. ♀ Cotton-top at PWP – Subord. ♀ Cotton-top at PWP	*Z*= 0.524, *P*= 1.000
			Subord. ♀ Emperor at TZ – Subord. ♀ Cotton-top at PWP	*Z*= 0.890, *P*= 1.000

III.3.2.1.2. Differences between study sites, species, and tamarin groups

Sample chemical richness was not significantly influenced by study site (**Table III.3**; **Fig. III.4a**), however it varied across species (**Table III.3**; **Fig. III.4b**; animal samples dataset). In particular, samples from black-headed spider monkeys contained more compounds (median: 92.5 ±SD 2.1) than samples from any other species (white-faced saki monkey: 19.0 ±0.0; silvery marmoset: 32.5 ±5.7; emperor tamarin: 18.0 ±10.7; and cotton-top tamarin: 18.0 ±8.3). However this difference was not verifiable statistically, since only two samples were available for black-headed spider monkeys, and one for white-faced saki monkeys. Samples from silvery marmosets contained significantly more compounds than those from emperor tamarins (**Table III.3; Fig. III.4b**). No difference in chemical richness was observed between the two tamarin species, nor with white-faced saki monkeys (**Table III.3; Fig. III.4b**). Moreover, chemical richness differed between tamarin groups (**Table III.3**; **Fig. III.4c**; tamarin samples dataset). Samples from emperor tamarins at TZ contained significantly more compounds than those from emperor tamarins at DMP (**Table III.3; Fig. III.4c**).

III.3.2.1.3. Differences between sex, reproductive status, and individual adult female tamarins

Sample chemical richness did not significantly differ between male and female samples (**Table III.3**; **Fig. III.5a**; tamarin samples dataset). Similarly, no difference was observed between the chemical richness of samples from reproductive and non-reproductive individuals (**Table III.3; Fig. III.5b**). However, sample chemical richness significantly differed between the reproductive emperor tamarin female at DMP and the reproductive emperor tamarin female at TZ (**Table III.3; Fig. III.5c**).

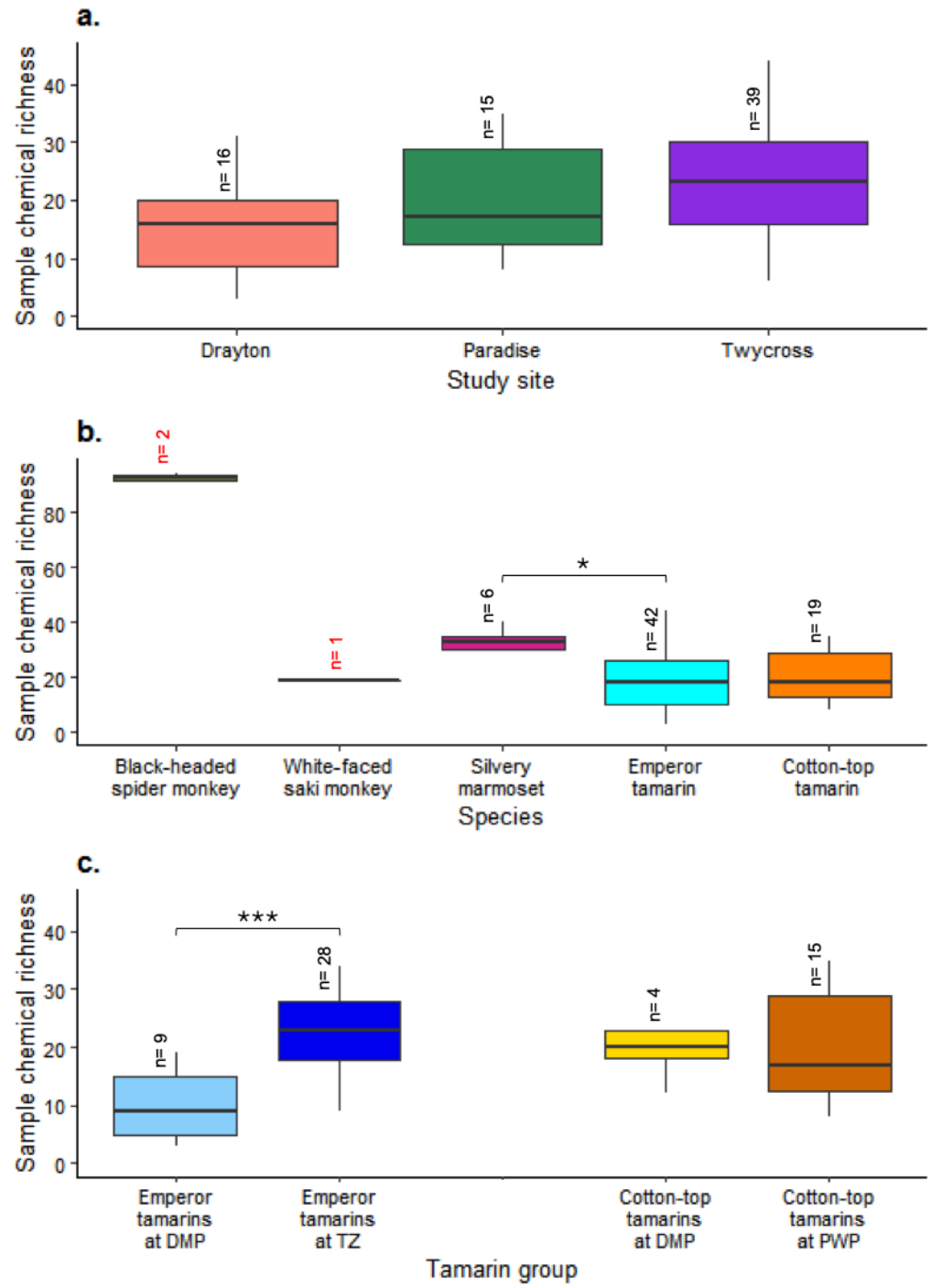


Figure III.4. Tukey's box-and-whiskers plots showing differences in sample chemical richness between **a.** study sites (animal samples dataset); **b.** primate species (animal samples dataset); and **c.** tamarin groups (tamarin samples dataset). Boxes indicate the median and interquartile range (IQR); whiskers give the smallest value ≥ lower hinge -1.5*IQR, and largest value ≤ upper hinge +1.5*IQR. n= sample size. Asterisks indicate statistically significant differences in pairwise Dunn's post-hoc tests for categories of n> 2 (✶ $P \leq 0.05$, ✶✶ $P \leq 0.01$, ✶✶✶ $P \leq 0.001$; **Table III.3**).

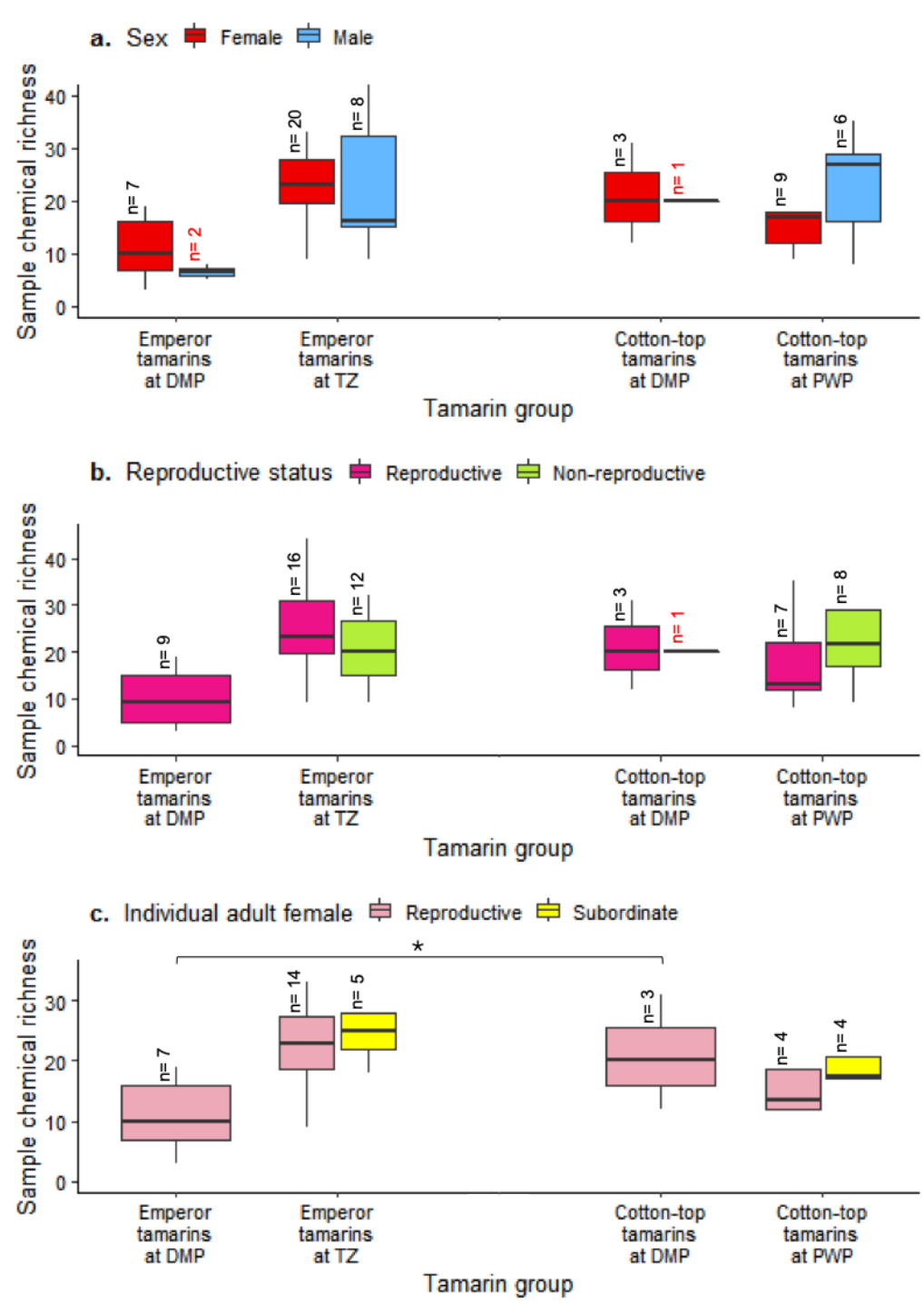


Figure III.5. Tukey's box-and-whiskers plots showing differences in sample chemical richness between **a.** males and females (tamarin samples dataset); **b.** reproductive and non-reproductive individuals (tamarin samples dataset); and **c.** reproductive and non-reproductive adult female tamarins (adult female tamarin samples dataset). Boxes indicate the median and interquartile range (IQR); whiskers give the smallest value ≥ lower hinge - 1.5*IQR, and largest value ≤ upper hinge +1.5*IQR. n= sample size. Asterisks indicate statistically significant differences in pairwise Dunn's post-hoc tests for categories of n> 2 (★ $P \leq 0.05$, ★★ $P \leq 0.01$, ★★★ $P \leq 0.001$; **Table III.3**).

III.3.2.1.4. Linear model including all factors

In addition, a generalized linear mixed model assessed the variation in sample chemical richness across all categories tested, using the tamarin samples dataset for which the number of samples per category was most balanced. The model included the fixed effects species, sex, reproductive status, sample type, and their interactions, as well as the random effects group and individual nested into group. Only sample type had a significant effect (**Table III.4**).

Table III.4. Results of best-fit generalized linear mixed model (*Poisson* family, log link function), testing the variation in sample chemical richness across categories of species, sex, reproductive status, sample type, and their interactions (tamarin samples dataset). Group, and individual nested into group, were included as random effects in the model. *P*-values were significant at $P \leq 0.05$ (in bold). SE: standard error of the mean, SD: standard deviation.

Fixed effects	**Paired comparisons**	**Estimate (±SE)**	***Z*-statistic**	***P*-value**
(intercept)		2.933 (± 0.337)	8.701	**< 0.001**
Species	Emperor – Cotton-top	0.040 (± 0.476)	0.084	0.933
Sex	Female – Male	0.062 (± 0.227)	0.274	0.784
Repro. status	Repro. – Non-repro.	-0.381 (± 0.236)	-1.617	0.106
Type	Scent-mark – Urine	-0.522 (± 0.111)	-4.694	**< 0.001**
Species : Sex	Cotton-top : Female – Male	0.344 (± 0.260)	1.325	0.185
Species : Repro. status	Cotton-top : Repro. – Non-repro.	0.522 (± 0.282)	1.853	0.064
Species : Type	Cotton-top : Scent-mark – Urine	-0.115 (± 0.175)	-0.657	0.511
Sex : Repro. status	Male : Repro. – Non-repro.	-0.264 (± 0.267)	-0.991	0.325
Sex : Type	Male : Scent-mark – Urine	0.141 (± 0.169)	0.836	0.403
Repro. Status : Type	Non-repro. : Scent-mark – Urine	0.292 (± 0.180)	1.621	0.105
Random effects		**Variance (±SD)**		
Group		0.184 (± 0.428)		
Group : Individual		0.035 (± 0.186)		

III.3.2.2. Variation in sample chemical diversity

III.3.2.2.1. Differences between sample types

To investigate variation in chemical diversity (i.e. the combination of individual compounds in a sample) between the different types of sample collected, NMDS was first applied to the all samples dataset. **Fig. III.6a** shows partial discrimination between sample types, in particular between animal samples (i.e. scent-marks, scent-gland swabs, and urine) and the 'background smell' (i.e. air and branch samples), most visible on the x–y and y–z dimensions (left and middle plots). However, homogeneity of multivariate dispersion between sample types was not validated for this dataset (**Table III.5**), preventing the use of PerMANOVA to further evaluate the significance of the observed variation.

To further assess dissimilarity between the chemical diversity of scent-mark, scent-gland secretion, and urine samples, the animal samples dataset was then used, for which homogeneity of multivariate dispersion for sample type was validated (**Table III.5**). **Fig. III.6b** shows partial discrimination between the chemical composition of scent-marks, scent-gland secretions, and urine, which were statistically significant (**Table III.5**). In addition, individual pairwise PerMANOVA tests confirmed a significant difference in chemical diversity between scent-mark and scent-gland samples (**Table III.5**).

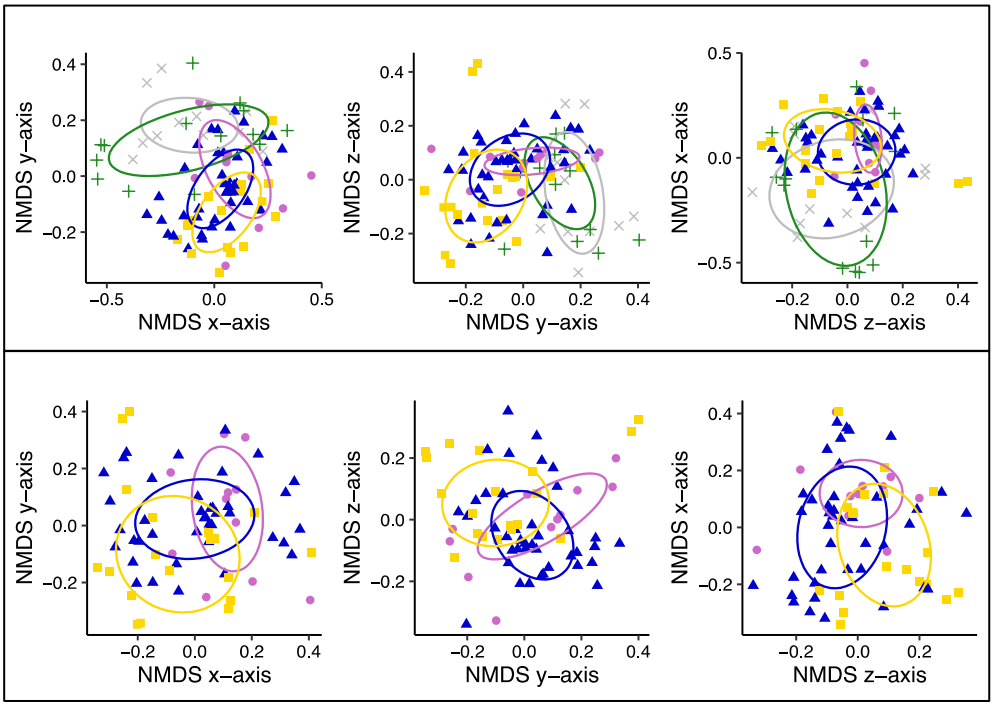


Figure III.6. Non-metric multidimensional scaling (NMDS) plots of scent samples showing similarity in chemical composition between **a.** samples of deposited scent-marks (▲), scent-gland secretion (●), urine (■), ambient air (×), and branch (+)(all samples dataset; 3D stress=0.16); and **b.** only samples of deposited scent-mark, scent-gland secretion, and urine (animal samples dataset; 3D stress=0.15). The three plots correspond to the same three-dimensional cloud of points, plotted on each dimension (x–y, y–z, and z–x). Ellipses represent the 50% confidence interval for each group.

Table III.5. Results of independent tests of homogeneity of multivariate dispersion for each group tested. When these were not significant (i.e. the group was homogenous), a permutational multivariate analysis of variance (PerMANOVA) was conducted to assess the importance of each group in explaining the differences in sample chemical diversity, followed by pairwise PerMANOVAs for categories of more than two levels. *F*= pseudo-*F* statistic; *df*= degrees of freedom; *res.df*= residual degrees of freedom; R_2= proportion of residual variance explained; *P*= *P*-value, and P_{adj}= *P*-value with Bonferroni adjustment, significant at *P*≤ 0.05 (in bold).

Dataset used	**Category tested**	**Homogeneity of multivariate dispersion ($F_{df, res.df}$, *P*)**	**PerMANOVA ($F_{df, res.df}$, R_2, *P*)**	**Pairwise comparisons**	**Pairwise PerMANOVA (*F*, R_2, P_{adj})**
All samples dataset	Sample type	$F_{4,90}$= 3.709, ***P*= 0.010**	NA		
Animal samples dataset	Sample type	$F_{2,67}$= 1.721, *P*= 0.220	$F_{2,67}$= 2.469, R_2= 0.069, ***P*= 0.001**	Scent-gland – Scent-mark	*F*= 2.903, R_2= 0.056, P_{adj}**= 0.003**
				Scent-gland – Urine	*F*= 2.810, R_2= 0.091, P_{adj}**= 0.003**
				Scent-mark – Urine	*F*= 1.940, R_2= 0.033, P_{adj}**= 0.015**
	Study site	$F_{2,67}$= 0.908, *P*= 0.420	$F_{2,67}$= 5.170, R_2= 0.134, ***P*= 0.001**	DMP – PWP	*F*= 3.616, R_2= 0.111, P_{adj}**= 0.003**
				DMP – TZ	*F*= 5.220, R_2= 0.090, P_{adj}**= 0.003**
				PWP – TZ	*F*= 6.060, R_2= 0.104, P_{adj}**= 0.003**
	Species	$F_{4,65}$= 16.46, ***P*= 0.010**	NA		

Table III.5. Continued (1/2).

Dataset used	Category tested	Homogeneity of multivariate dispersion ($F_{df, res.df}$, P)	PerMANOVA ($F_{df, res.df}$, R_2, P)	Pairwise comparisons	Pairwise PerMANOVA (F, R_2, P_{adj})
Tamarin samples dataset	Species	$F_{1,54}$= 0.233, P= 0.660	$F_{1,54}$= 4.427, R_2= 0.076, **P= 0.001**		
	Group	$F_{3,52}$= 0.860, P= 0.550	$F_{3,52}$= 4.129, R_2= 0.192, **P= 0.001**	Emperor at DMP – Emperor at TZ	F= 5.140, R_2= 0.128, **P_{adj}= 0.006**
				Emperor at DMP – Cotton-top at DMP	F= 1.601, R_2= 0.127, P_{adj}= 0.282
				Emperor at DMP – Cotton-top at PWP	F= 3.552, R_2= 0.139, **P_{adj}= 0.006**
				Cotton-top at DMP – Emperor at TZ	F= 2.642, R_2= 0.081, **P_{adj}= 0.006**
				Cotton-top at DMP – Cotton-top at PWP	F= 2.374, R_2= 0.123, **P_{adj}= 0.012**
				Cotton-top at PWP – Emperor at TZ	F= 6.213, R_2= 0.132, **P_{adj}= 0.006**
	Sex	$F_{1,54}$= 3.717, P= 0.080	$F_{1,54}$= 1.640, R_2= 0.029, **P= 0.024**		
	Repro. status	$F_{1,54}$= 0.599, P= 0.510	$F_{1,54}$= 1.970, R_2= 0.035, **P= 0.001**		

Table III.5. Continued (2/2).

Dataset used	Category tested	Homogeneity of multivariate dispersion ($F_{df,\ res.df}$, P)	PerMANOVA ($F_{df,\ res.df}$, R_2, P)	Pairwise comparisons	Pairwise PerMANOVA (F, R_2, P_{adj})
Adult female tamarin samples dataset	Female identity	$F_{5,31}$= 0.458, P= 0.770	$F_{5,31}$= 2.034, R_2= 0.247, **P= 0.001**	Repro. ♀ Emperor at DMP – Repro. ♀ Emperor at TZ	F= 3.228, R_2= 0.145, **P_{adj}= 0.015**
				Repro. ♀ Emperor at DMP – Repro. ♀ Cotton-top at DMP	F= 1.294, R_2= 0.139, P_{adj}= 1.000
				Repro. ♀ Emperor at DMP – Repro. ♀ Cotton-top at PWP	F= 2.139, R_2= 0.192, P_{adj}= 0.150
				Repro. ♀ Emperor at DMP – Subord. ♀ Emperor at TZ	F= 3.035, R_2= 0.233, P_{adj}= 0.075
				Repro. ♀ Emperor at DMP – Subord. ♀ Cotton-top at PWP	F= 2.268, R_2= 0.201, P_{adj}= 0.060
				Repro. ♀ Emperor at TZ – Repro. ♀ Cotton-top at DMP	F= 1.780, R_2= 0.106, P_{adj}= 0.240
				Repro. ♀ Emperor at TZ – Repro. ♀ Cotton-top at PWP	F= 2.053, R_2= 0.114, **P_{adj}= 0.045**
				Repro. ♀ Emperor at TZ – Subord. ♀ Emperor at TZ	F= 0.774, R_2= 0.044, P_{adj}= 1.000
				Repro. ♀ Emperor at TZ – Subord. ♀ Cotton-top at PWP	F= 2.157, R_2= 0.119, **P_{adj}= 0.045**
				Repro. ♀ Cotton-top at DMP – Repro. ♀ Cotton-top at PWP	F= 1.959, R_2= 0.282, P_{adj}= 0.450
				Repro. ♀ Cotton-top at DMP – Subord. ♀ Emperor at TZ	F= 2.115, R_2= 0.261, P_{adj}= 0.165
				Repro. ♀ Cotton-top at DMP – Subord. ♀ Cotton-top at PWP	F= 1.791, R_2= 0.264, P_{adj}= 0.795
				Repro. ♀ Cotton-top at PWP – Subord. ♀ Emperor at TZ	F= 2.261, R_2= 0.244, P_{adj}= 0.180
				Repro. ♀ Cotton-top at PWP – Subord. ♀ Cotton-top at PWP	F= 0.841, R_2= 0.123, P_{adj}= 1.000
				Subord. ♀ Emperor at TZ – Subord. ♀ Cotton-top at PWP	F= 2.268, R_2= 0.245, P_{adj}= 0.150

III.3.2.2.2. Differences between study sites, species, and tamarin groups

Differences in sample chemical diversity between study sites was assessed using the animal samples dataset, for which homogeneity of multivariate dispersion for study sites was validated (**Table III.5**). **Fig. III.7** shows a good discrimination between the three study sites, in particular on the x–z dimension (right plot). This suggests a strong effect of study site on sample chemical diversity, further confirmed by the result of the PerMANOVA (**Table III.5**). Moreover, pairwise PerMANOVA tests confirmed a significant difference in sample chemical diversity between all three sites (**Table III.5**).

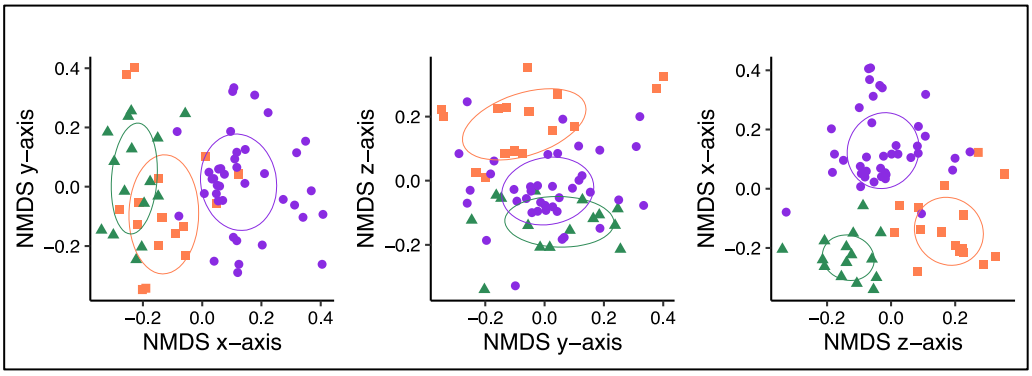


Figure III.7. NMDS plots of scent samples showing similarity in sample chemical composition between the three study sites: Drayton Manor Park (■), Twycross Zoo (●), and Paradise Wildlife Park (▲) (animal samples dataset; 3D stress=0.16). The three plots correspond to the same three-dimensional cloud of points, plotted on each dimension (x–y, y–z, and z–x). Ellipses represent the 50% confidence interval for each group.

In order to compare sample chemical diversity between species, the animal samples dataset was first used. **Fig. III.8a** shows partial discrimination between species, in particular between the three callitrichid species (i.e. silvery marmosets, and emperor and cotton-top tamarins) and the two non-callitrichid species (i.e. black-headed spider monkeys and white-faced saki monkeys). Homogeneity of variance in the species category was not validated for this dataset (**Table III.5**), owing to the limited sample size for silvery marmosets, black-headed spider monkeys and white-faced saki monkeys. To further assess differences of chemical diversity between the two tamarin species, which are the focus of the present study and for which most samples were collected, the tamarin

sample dataset was then employed (**Table III.2**). Homogeneity of multivariate dispersion for species was validated for this dataset and the difference observed was significant (**Table III.5**). **Fig. III.8b** shows good discrimination between the chemical composition of samples from emperor and cotton-top tamarins, especially on the x–y and z–x dimensions (left and right plots).

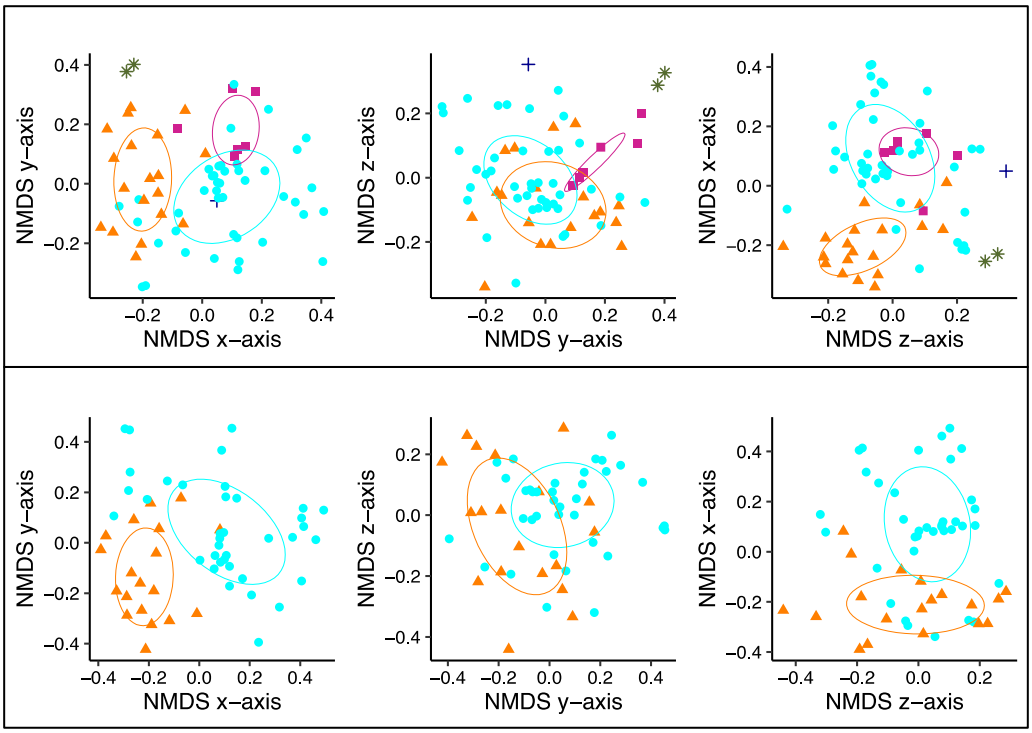


Figure III.8. NMDS plots of scent samples showing similarity in sample chemical composition between **a.** all five primate species: emperor tamarins, *S. imperator* (●), cotton-top tamarins, *S. oedipus* (▲), silvery marmosets, *M. argentatus* (■), white-faced saki monkeys, *P. pithecia* (+), and black-headed spider monkeys, *A. fusciceps* (✳) (animal samples dataset; 3D stress=0.16); and **b.** the two tamarin species (tamarin samples dataset; 3D stress=0.14). The three plots correspond to the same three-dimensional cloud of points, plotted on each dimension (x–y, y–z, and z–x). Ellipses represent the 50% confidence interval for groups composed of at least four samples.

In addition, differences in sample chemical diversity between tamarin groups was assessed using the tamarin samples dataset, for which homogeneity of multivariate dispersion was validated. The difference between groups was significant (**Table III.5**). The four groups appear well clustered on the NMDS plots, especially on the x–y dimension (left plot; **Fig. III.9**). In particular, the emperor tamarin group at TZ appeared well

differentiated from the others. Moreover, pairwise PerMANOVA tests were significant between all pairs of tamarin groups, except between emperor and cotton-top tamarins at DMP (**Table III.5**).

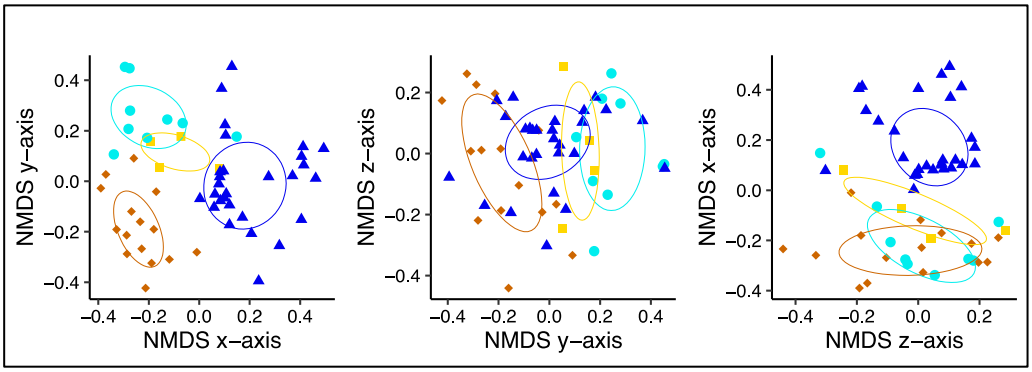


Figure III.9. NMDS plots of scent samples showing similarity in sample chemical composition between the four tamarin groups studied: emperor tamarins at DMP (●), emperor tamarins at TZ (▲), cotton-top tamarins at DMP (■), and cotton-top tamarins at PWP (♦) (tamarin samples dataset; 3D stress=0.14). The three plots correspond to the same three-dimensional cloud of points, plotted on each dimension (x–y, y–z, and z–x). Ellipses represent the 50% confidence interval for each group.

III.3.2.2.3. Differences between sex, reproductive status, and individual adult female tamarins

Sex differences in sample chemical diversity was assessed using the tamarin dataset, for which homogeneity of multivariate dispersion was validated (**Table III.5**). Similarly to results of chemical richness, NMDS did not show discrimination between the chemical diversity of male and female tamarin samples (**Fig. III.10**). Nevertheless, difference in chemical diversity for sex was significant in the PerMANOVA analysis, although the proportion of residual variance explained and *pseudo-F* statistic were low, and the associated *P*-value high, compared with the previous categories tested (**Table III.5**). This likely indicates a smaller effect of sex and would explain why samples from male and female tamarins were not discriminated on the NMDS plots.

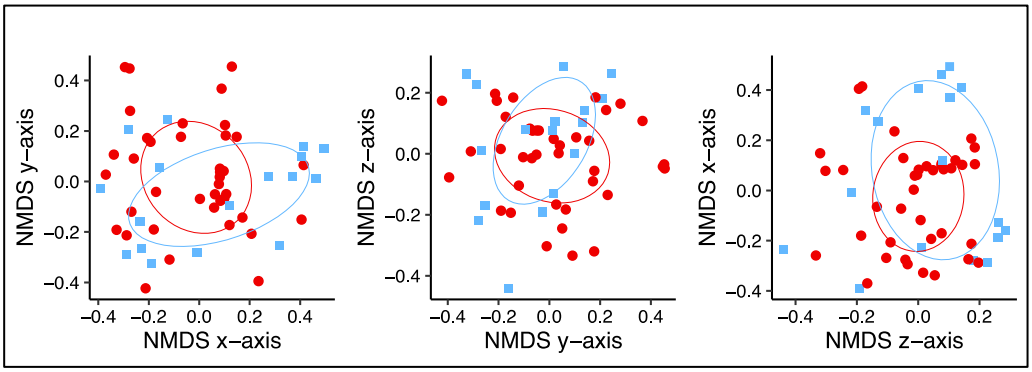


Figure III.10. NMDS plots of scent samples showing similarity in chemical composition between male (■) and female (●) tamarin samples (tamarin samples dataset; 3D stress=0.14). The three plots correspond to the same three-dimensional cloud of points, plotted on each dimension (x–y, y–z, and z–x). Ellipses represent the 50% confidence interval for each group.

To investigate differences in the chemical diversity of samples from reproductive and non-reproductive tamarins, the tamarin samples dataset was used. Homogeneity of multivariate dispersion for reproductive status was validated, and the difference observed was significant (**Table III.5**). **Fig. III.11** shows partial discrimination between samples of reproductive and non-reproductive individuals of the two tamarin species. As for sex, the proportion of residual variance explained and *pseudo-F* statistic were lower than that of the previous categories tested, which reflects the absence of complete discrimination between reproductive and non-reproductive tamarins on the NMDS plots (**Table III.5**).

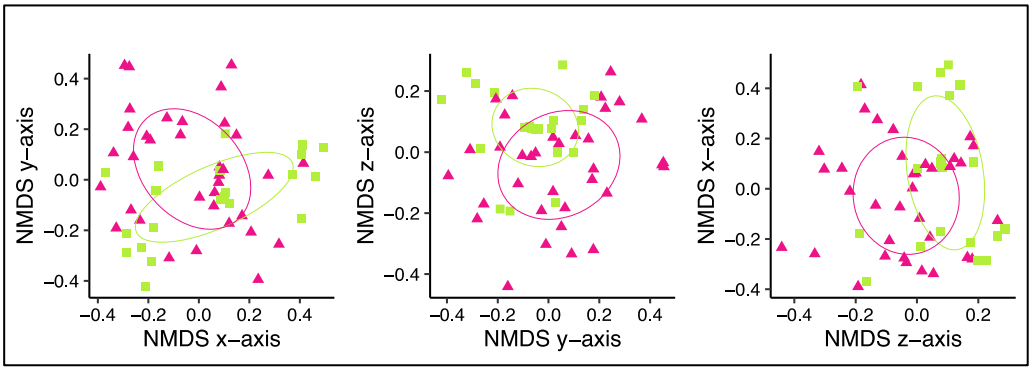


Figure III.11. NMDS plots of scent samples showing similarity in sample chemical composition between reproductive (▲) and non-reproductive (■) tamarins of both sexes (tamarin samples dataset; 3D stress=0.14). The three plots correspond to the same three-dimensional cloud of points, plotted on each dimension (x–y, y–z, and z–x). Ellipses represent the 50% confidence interval for each group.

It was only possible to collect multiple scent-mark samples from a few individuals included in this study. Therefore, chemical differences at the individual level were only tested on six reproductive and non-reproductive adult female tamarins, for which at least three samples were collected. Using the adult female tamarin samples dataset, homogeneity of multivariate dispersion for the individual was validated, and the difference between individual female tamarins was significant (**Table III.5**). **Fig. III.12** shows partial discrimination between samples of the six females included in the analysis. In particular, pairwise differences in chemical diversity between individual females were significant between the reproductive female emperor tamarin at DMP and the reproductive female emperor tamarin at TZ, and between the reproductive female emperor tamarin at TZ and both the reproductive and non-reproductive females cotton-top tamarin at PWP (**Table III.5**), reflected in the NMDS plots (**Fig. III.12**).

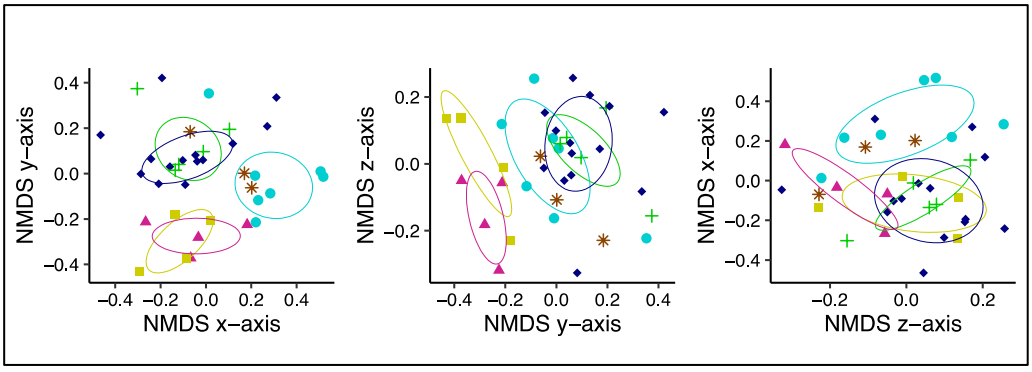


Figure III.12. NMDS plots of scent samples showing similarity in sample chemical composition between six adult female tamarins: reproductive female emperor tamarin at DMP (●), reproductive female emperor tamarin at TZ (♦), reproductive female cotton-top tamarin at DMP (✱), reproductive female cotton-top tamarin at PWP (▲), non-reproductive female emperor tamarin at TZ (+), and non-reproductive female cotton-top tamarin at PWP (■) (female tamarin samples dataset; 3D stress: 0.13). The three plots correspond to the same three-dimensional cloud of points, plotted on each dimension (x–y, y–z, and z–x). Ellipses represent the 50% confidence interval for groups composed of at least four samples.

III.3.2.2.4. Multifactorial PerMANOVA including all factors

A multifactorial PerMANOVA using the tamarin samples dataset assessed the relative effect of all the sample categories on sample chemical diversity. This model accounted for multicollinearity between

factors, where sex, reproductive status and individual were included as terms nested into group, and site was not included as it was redundant with species and group. All the factors, except for individual, had a significant effect on the dissimilarity of sample chemical diversity (**Table III.6**). Species and group had the greatest effect, as indicated by the highest *Pseudo-F* statistics in **Table III.6**.

Table III.6. Results of a PerMANOVA testing the effect of sample type, species, group, sex, reproductive status (nested in group), and individual (nested in group), on sample chemical similarity. *df*= degrees of freedom, *SSoS*= sequential sum of squares, *MS*= mean squares, *Pseudo-F*= *pseudo-F* statistic, R_2= proportion of residual variance explained, and *P*= *P*-value (significant at *P*≤ 0.05, in bold).

Effect tested	*df*	*SSoS*	*MS*	*Pseudo-F*	R_2	*P*
Sample type	1	0.675	0.675	2.401	0.035	**0.003**
Species	1	1.475	1.475	5.244	0.076	**0.001**
Group	2	2.212	1.106	3.933	0.114	**0.001**
Sex	1	0.757	0.575	2.044	0.030	**0.003**
Group : Repro. status	3	1.117	0.372	1.324	0.058	**0.022**
Group : Individual	8	2.395	0.299	1.065	0.123	0.226
Residuals	39	10.967	0.281		0.565	
Total	55	19.415			1.000	

III.3.3. Identification of compounds in callitrichid scent samples

III.3.3.1. Identifty of compounds

The automated NIST mass spectral library provided putative identities to all 407 compounds retrieved from the samples (see **Appendix C**, **Table C.1**). Nevertheless, in most cases this identification was not trustworthy unless the identity match was very high (≥80%), notably owing to the fact that the chromatogram peaks, many of which might have very similar retention times, were often not fully resolved, hence their mass spectra may have contained fragments from more than one compound. This was especially a problem for peaks of low relative abundance. In

addition, synthetic organic compounds were the main candidate identities given by the NIST library. Synthetic organic compounds are widely used in industry, which is the primary user of the NIST library. However, all authentic compounds originating from the samples were naturally occurring organic compounds, many of which may be unregistered on the NIST database. For these reasons, a subset of 47 compounds was selected, for which the NIST library identity was individually verified by visual inspection of the peaks' mass spectra and retention times (i.e. compounds #01–#47; **Table III.7**). These 47 compounds of interest were also chosen given their prevalence in the samples, and/or their prior mention in the mammalian semiochemistry literature. The same compound retrieved from different samples did generally not show identical retention times, as in chromatography retention times commonly vary slightly between runs. Therefore, values of compound average retention time and associated standard deviation were used instead, as presented in **Table III.7**.

Whenever possible, the identity of a compound was confirmed, or refuted, by comparison of its retention time with that of the commercially obtained compound analysed under the same conditions. As a result, 22 compounds were positively identified (marked with an asterisk in **Table III.7**). Since authentic compounds and corresponding commercially obtained compounds were not co-injected, their retention times usually differed slightly, as retention times may vary between GC-MS runs. Hence, compound identity was considered validated if **1.** the mass spectra of the compound in the sample (**Fig. III.13a**), and that of the commercially obtained compound (**Fig. III.13b**), were closely matched; and **2.** the retention time of the compound in the sample fell within the width of the genuine compound's peak at mid-height (**Fig. III.13c**); which was visually assessed. In some cases, identification was less certain. For example, commercially obtained samples of butanoic acid, pentanoic acid, tridecane, and hexadecane, eluted a short time after the corresponding compounds in the samples, suggesting that the compounds in the secretion were branched-chain variants of same molecular weight as the commercially obtained compounds (**Table III.7**).

Table III.7. Subset of 47 compounds retrieved from the samples, selected as compounds of interest given their prevalence in the samples, and/or their prior mention in the mammalian semiochemistry literature. Putative identity of the compounds was established by mass spectral library search, then verified by visual inspection of the mass spectra and retention times (RT). Identity of the 22 compounds marked with an asterisk (⁎) was confirmed by comparison of their retention times with those of commercially obtained compounds. Diagrams of the compounds' chemical structure are reproduced from the NIST chemistry WebBook (www.webbook.nist.gov). SD= standard deviation.

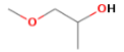
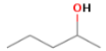
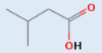
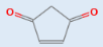
#	Mean RT ±SD (min)	Candidate compound identity	Functional group	Chemical structure	Prevalence†
01	3.04 ±0.01	Butan-1-ol⁎	Alcohol		11
02	3.19 ±0.04	Methoxypropan-2-ol	Multifunctional compound		8
03	3.34 ±0.01	Pentan-2-one⁎	Ketone		3
04	3.58 ±0.01	Pentan-2-ol⁎	Alcohol		5
05	4.70 ±0.06	Propane-1,2-diol	Alcohol		10
06	6.14 ±0.01	Hexanal⁎	Aldehyde		47
07	6.30 ±0.09	2-Methylpropanoic acid	Carboxylic acid		3
08	7.24 ±0.02	Furfural	Heteroaromatic aldehyde		14
09	7.93 ±0.03	3-Methylbutanoic acid	Carboxylic acid		3
10	8.05 ±0.02	2-Furanmethanol⁎	Alcohol		23
11	8.83 ±0.02	Cyclopent-2-en-1,4-dione	Ketone		5

Table III.7. Continued (1/3).

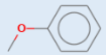
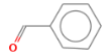
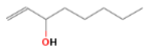
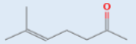
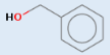
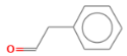
#	Mean RT ±SD (min)	Candidate compound identity	Functional group	Chemical structure	Prevalence†
12	8.99 ±0.00	1,2-Dimethylbenzene⋆	Aromatic hydrocarbon		2
13	9.15 ±0.01	Methylcycloheptanone	Ketone	Unk.	14
14	9.33 ±0.02	Heptanal⋆	Aldehyde		32
15	9.74 ±0.06	2,5-Dimethylpyrazine	Heteroaromatic compound		2
16	9.79 ±0.02	Anisole⋆	Aromatic ether		12
17	11.14 ±0.03	Benzaldehyde⋆	Aromatic aldehyde		54
18	11.33 ±0.00	1,3,5-Trimethylbenzene⋆	Aromatic hydrocarbon		3
19	11.78 ±0.00	1-Octen-3-ol⋆	Unsaturated alcohol		7
20	11.98 ±0.01	6-Methyl-5-hepten-2-one⋆	Unsaturated ketone		3
21	12.08 ±0.00	1,2,3- or 1,2,4-Trimethylbenzene⋆	Aromatic hydrocarbon		9
22	13.42 ±0.01	Benzyl alcohol⋆	Alcohol		3
23	13.56 ±0.03	Benzeneacetaldehyde⋆	Aldehyde		26
24	14.21 ±0.04	Acetophenone⋆	Aromatic ketone		36

Table III.7. Continued (2/3).

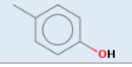
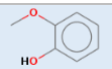
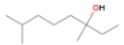
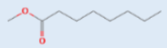
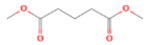
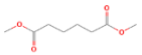
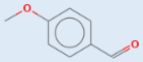
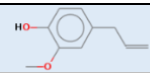
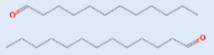
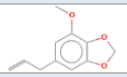
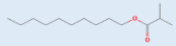
#	Mean RT ±SD (min)	Candidate compound identity	Functional group	Chemical structure	Prevalence†
25	14.58 ±0.05	p-Cresol⋆	Aromatic alcohol		41
26	14.70 ±0.01	p-Cymene	Aromatic hydrocarbon		5
27	14.85 ±0.04	2-Methoxyphenol	Multifunctional compound		29
28	15.07 ±0.02	3,7-Dimethyloctan-3-ol	Alcohol		15
29	15.71 ±0.07	Methyl octanoate	Ester		2
30	16.14 ±0.01	Dimethyl pentanedioate⋆	Ester		5
31	16.62 ±0.04	*trans*-1-Methyl-4-(1-methylethyl)cyclohexanol	Alcohol		26
32	17.02 ±0.01	1-Nonanol⋆	Alcohol		5
33	17.18 ±0.04	Branched C8 carboxylic acid	Carboxylic acid	Unk.	4
34	18.79 ±0.01	Dimethyl hexanedioate	Ester		3
35	19.10 ±0.07	4-Methoxybenzaldehyde⋆	Multifunctional compound		63
36	19.43 ±0.03	Cyclodecane	Cycloalkane		30
37	19.99 ±0.00	Branched C13 alkane	Alkane	Unk.	10

Table III.7. Continued (3/3).

#	Mean RT ±SD (min)	Candidate compound identity	Functional group	Chemical structure	Prevalence†
38	21.48 ±0.01	Eugenol⋆	Multifunctional compound		4
39	21.54 ±0.07	3-Methyltridecane	Alkane		3
40	22.49 ±0.01	Do- or Tri-decanal	Aldehyde		11
41	23.12 ±0.06	Diethylene glycol dibutyl ether	Ether		23
42	23.86 ±0.07	Cyclododecane	Cycloalkane		43
43	24.93 ±0.07	Myristicin	Multifunctional compound		18
44	25.25 ±0.01	2-Methyldecylpropanoate	Ester		4
45	26.27 ±0.07	Branched C16 alkane	Alkane	Unk.	31
46	27.46	Tetra- or Penta-decanal	Aldehyde		1
47	28.44 ±0.01	Heptadecane⋆	Alkane		3

† Prevalence is the number of samples containing the compound of interest.

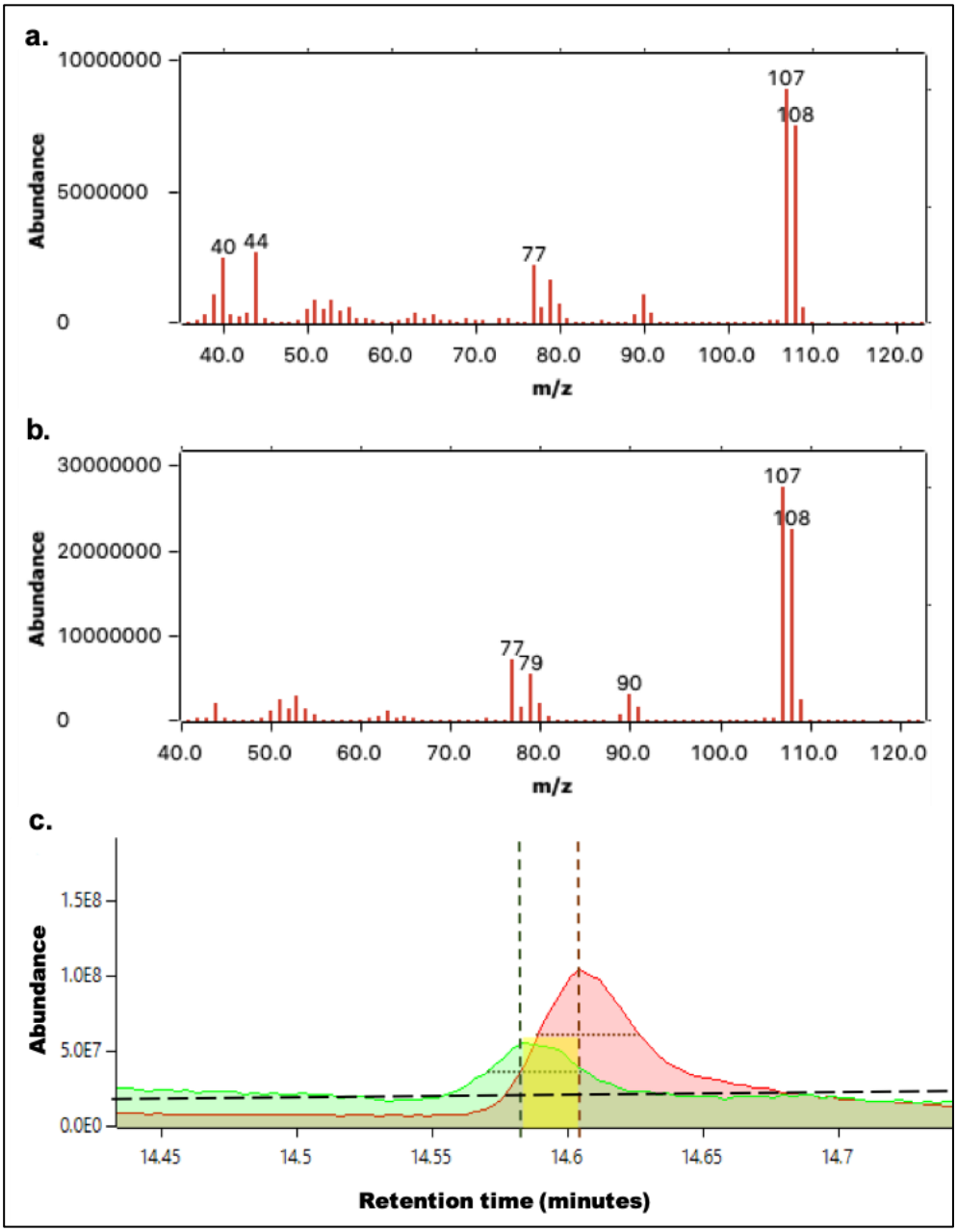


Figure III.13. Mass spectra of **a.** p-cresol retrieved from a sample, and **b.** commercially obtained p-cresol analysed under the same conditions, showing an almost identical ion pattern; **c.** overlaid chromatogram portions showing authentic p-cresol peak (in green), and commercially obtained p-cresol peak (in red). The horizontal black dashed line indicates the background threshold. Vertical dashed lines mark the peak apex, which corresponds to the compounds' retention time. Horizontal dotted lines mark the peak mid-height. The yellow shaded area indicates overlap in retention time of the two peaks, which means they can be considered as the same compound.

Over 60% of the compounds of interest retrieved from the samples were hydrocarbons (i.e. alkanes, cycloalkanes and aromatic hydrocarbons, 21.3%), alcohols (21.3%), and aldehydes (14.9%; **Fig. III.14**; **Table III.7**). Nearly 35% of these compounds contained an aromatic group, i.e. a benzene ring (e.g. benzaldehyde [#17]) or a furan ring (e.g. furfural [#08]); the rest were aliphatic, i.e. open-chain compounds, either straight (e.g. heptadecane [#47]), branched (e.g. pentan-2-ol [#04]), or cyclic compounds (e.g. cyclodecane [#36]).

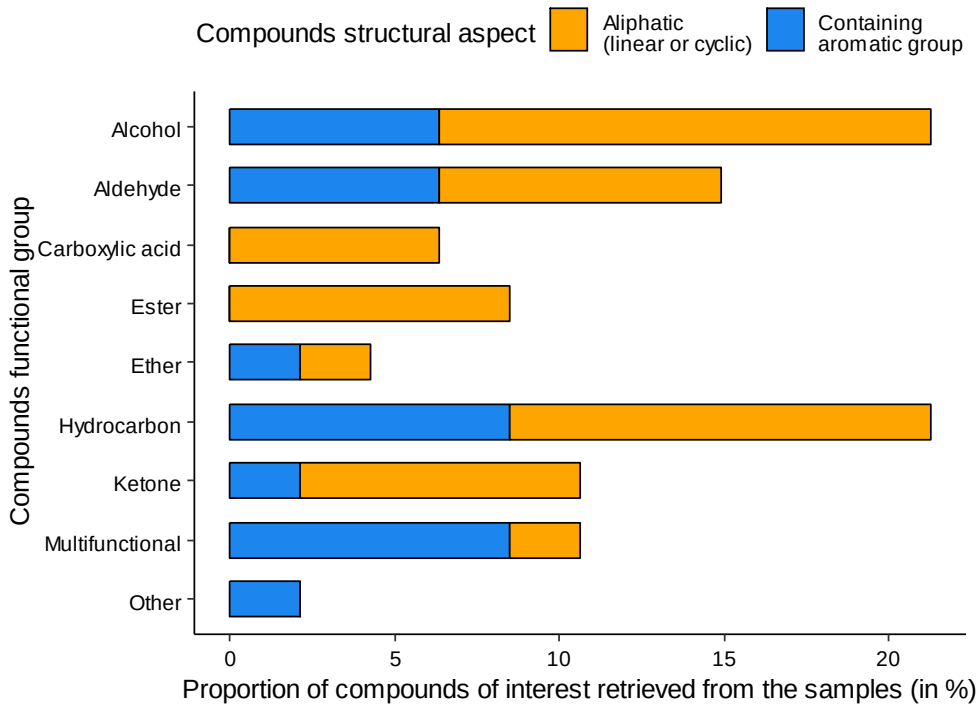


Figure III.14. Functional group and structural aspect (i.e. aliphatic or containing an aromatic group) of the 47 compounds of interest retrieved from the samples.

III.3.3.2. Occurrence of compounds of interest across the different categories of samples

III.3.3.2.1. Occurrence of compounds of interest in the different sample types and primate species studied

While some of the 47 compounds of interest identified in this study were ubiquitous, i.e. found in all five species sampled, and/or in both glandular secretions, deposited scent-marks, and urine, others appeared to be specific to a single sample type or species (**Table III.8**). In particular, hexanal (#06), benzaldehyde (#17), 4-methoxybenzaldehyde (#35), and

cyclododecane (#42) were retrieved from many samples of the three types, and from all five species of primates. Most compounds were found in all three types of samples (34.0%), or in both deposited scent-marks and urine (25.5%; animal samples dataset; **Fig. III.15**; **Table III.8**). The two multifunctional compounds methoxypropan-2-ol (#02) and 2,5-dimethylpyrazine (#15) were the only compounds solely found in glandular secretions. One aldehyde, tetra- or penta-decanal (#46); two esters, methyl octanoate (#29) and dimethyl hexanoate (#34); three hydrocarbons, 1,2-dimethylbenzene (#12), 1,3,5-trimethylbenzene (#18), and 3-methyltridecane (#39); and one ketone, cyclopent-2-en-1,4-dione (#11), were specific to scent-mark samples. Finally, no compound was specific to urine in this study (**Fig. III.15**; **Table III.8**).

Do- or tri-decanal (#40) and the branched C16 alkane (#45) were found in all species but black-headed spider monkeys; heptanal (#14), 1-octen-3-ol (#19), benzeneacetaldehyde (#23), and p-cresol (#25) in all species but white-faced saki monkeys, while 21.3% of the compounds were specific to the three callitrichids (**Fig. III.16**; **Table III.8**). In addition, an important proportion of compounds of interest (17.0%) were shared between the two tamarin species only: pentan-2-one (#03), cyclopent-2-en-1,4-dione (#11), anisole (#16), methyl octanoate (#29), dimethyl pentanedioate (#30), 1-nonanol (#32), dimethyl hexanoate (#34), cyclodecane (#36), eugenol (#38), and myristicin (#43). Moreover, in the present study 2-methylpropanoic acid (#07) and 3-methyltridecane (#39) were unique to emperor tamarins; 1,3,5- and 1,2,3-/1,2,4-trimethylbenzene (#18 and #21), 2-methyldecylpropanoate (#44), and tetra- or penta-decanal (#46) to cotton-top tamarins. However, no compound was unique to saki or spider monkeys, maybe owing to the fact that only one, and two samples, respectively, of these species were included in the animal dataset. Similarly, 2,5-dimethylpyrazine (#15) was the only compound specific to silvery marmosets; however the species was represented in the present dataset by only six samples of suprapubic scent-gland secretion, which may explain the absence of other compounds unique to silvery marmosets (**Fig. III.16**; **Table III.8**).

Table III.8. Occurrence of the 47 compounds of interest in the five primate species included in the study, retrieved from suprapubic scent-gland secretions (**G**, collected for emperor tamarins and silvery marmosets), deposited scent-marks (**S**, collected for emperor and cotton-top tamarins, and white-faced saki monkeys), and urine (**U**, collected for emperor and cotton-top tamarins, and black-headed spider monkeys), and their prevalence in the samples (numbers in brackets). Candidate compound identities verified by comparison with authentic compounds are marked with an asterisk (⋆), the other names are tentative identities.

Compounds functional group	Compound identity (#)	Primate species				
		Emperor tamarin	Cotton-top tamarin	Silvery marmoset	White-faced saki monkey	Black-headed spider monkey
Alcohol	Butan-1-ol⋆ (#01)	G(1) S(3)	S(1)	G(6)	-	-
	Pentan-2-ol⋆ (#04)	S(1) U(1)	S(1) U(1)	-	S(1)	-
	Propane-1,2-diol (#05)	G(3) S(3)	-	G(4)	-	-
	2-Furanmethanol⋆ (#10)	G(1) S(10)	S(7) U(1)	G(4)	-	-
	1-Octen-3-ol⋆ (#19)	S(1)	S(2) U(3)	-	-	U(1)
	Benzyl alcohol⋆ (#22)	G(1) S(1) U(1)	-	-	-	-
	p-Cresol⋆ (#25)	G(3) S(12) U(3)	S(13) U(6)	G(3)	-	U(1)
	3,7-Dimethyloctan-3-ol (#28)	G(1) S(6)	S(2)	G(6)	-	-
	trans-1-Methyl-4-(1-methylethyl)cyclohexanol (#31)	S(18) U(5)	-	G(3)	-	-
	1-Nonanol⋆ (#32)	S(1) U(2)	S(2)	-	-	-
Aldehyde (1/2)	Hexanal⋆ (#06)	G(3) S(16) U(6)	S(10) U(3)	G(6)	S(1)	U(2)
	Furfural (#08)	S(6)	S(2) U(1)	G(5)	-	-
	Heptanal⋆ (#14)	S(13) U(1)	S(7) U(3)	G(6)	-	U(2)

Table III.8. Continued (1/2).

Compounds functional group	Compound identity (#)	Primate species				
		Emperor tamarin	Cotton-top tamarin	Silvery marmoset	White-faced saki monkey	Black-headed spider monkey
Aldehyde (2/2)	Benzaldehyde⋆ (#17)	G(2) S(20) U(10)	S(7) U(6)	G(6)	S(1)	U(2)
	Benzeneacetaldehyde⋆ (#23)	G(1) S(7) U(5)	S(5) U(1)	G(5)	-	U(2)
	Do- or Tri-decanal (#40)	S(2) U(1)	S(3) U(2)	G(2)	S(1)	-
	Tetra- or Penta-decanal (#46)	-	S(1)	-	-	-
Carboxylic acid	2-Methylpropanoic acid (#07)	S(1) U(2)	-	-	-	-
	3-Methylbutanoic acid (#09)	G(1) S(1)	-	-	S(1)	-
	Branched C8 carboxylic acid (#33)	G(1) U(1)	U(1)	G(1)	-	-
Ester	Methyl octanoate (#29)	S(1)	S(1)	-	-	-
	Dimethyl pentanedioate⋆ (#30)	S(2)	S(3)	-	-	-
	Dimethyl hexanedioate (#34)	S(2)	S(1)	-	-	-
	2-Methyldecylpropanoate (#44)	-	S(3) U(1)	-	-	-
Ether	Anisole⋆ (#16)	S(5) U(5)	S(1) U(1)	-	-	-
	Diethyleneglycol dibutyl ether (#41)	G(3) S(11) U(6)	-	G(3)	-	-
Hydrocarbon (1/2)	1,2-Dimethylbenzene⋆ (#12)	-	S(1)	-	S(1)	-
	1,3,5-Trimethylbenzene⋆ (#18)	-	S(3)	-	-	-
	1,2,3- or 1,2,4-Trimethylbenzene⋆ (#21)	-	S(7) U(2)	-	-	-
	p-Cymene (#26)	-	S(3)	-	-	U(2)

Table III.8. Continued (2/2).

Compounds functional group	Compound identity (#)	Primate species				
		Emperor tamarin	Cotton-top tamarin	Silvery marmoset	White-faced saki monkey	Black-headed spider monkey
Hydrocarbon (2/2)	Cyclodecane (#36)	S(15) U(4)	S(9) U(2)	-	-	-
	Branched C13 alkane (#37)	S(6)	S(2)	G(2)	-	-
	3-Methyltridecane (#39)	S(3)	-	-	-	-
	Cyclododecane (#42)	G(4) S(18) U(8)	S(3) U(2)	G(5)	S(1)	U(2)
	Branched C16 alkane (#45)	S(17) U(5)	S(3)	G(5)	S(1)	-
	Heptadecane⋆ (#47)	-	S(2)	G(1)	-	-
Ketone	Pentan-2-one⋆ (#03)	S(1) U(1)	S(1)	-	-	-
	Cyclopent-2-en-1,4-dione (#11)	S(4)	S(1)	-	-	-
	Methylcycloheptanone (#13)	G(1) S(6)	S(3) U(1)	G(3)	-	-
	6-Methyl-5-hepten-2-one⋆ (#20)	S(1)	-	G(2)	-	-
	Acetophenone⋆ (#24)	G(1) S(19) U(4)	S(5) U(1)	G(6)	-	-
Multifunctional /Other	Methoxypropan-2-ol (#02)	G(3)	-	G(5)	-	-
	2,5-Dimethylpyrazine (#15)	-	-	G(2)	-	-
	2-Methoxyphenol (#27)	S(10) U(3)	S(10) U(4)	-	-	U(2)
	4-Methoxybenzaldehyde⋆ (#35)	G(3) S(24) U(10)	S(13) U(6)	G(4)	S(1)	U(2)
	Eugenol⋆ (#38)	S(2)	S(1) U(1)	-	-	-
	Myristicin (#43)	S(11) U(1)	S(6)	-	-	-

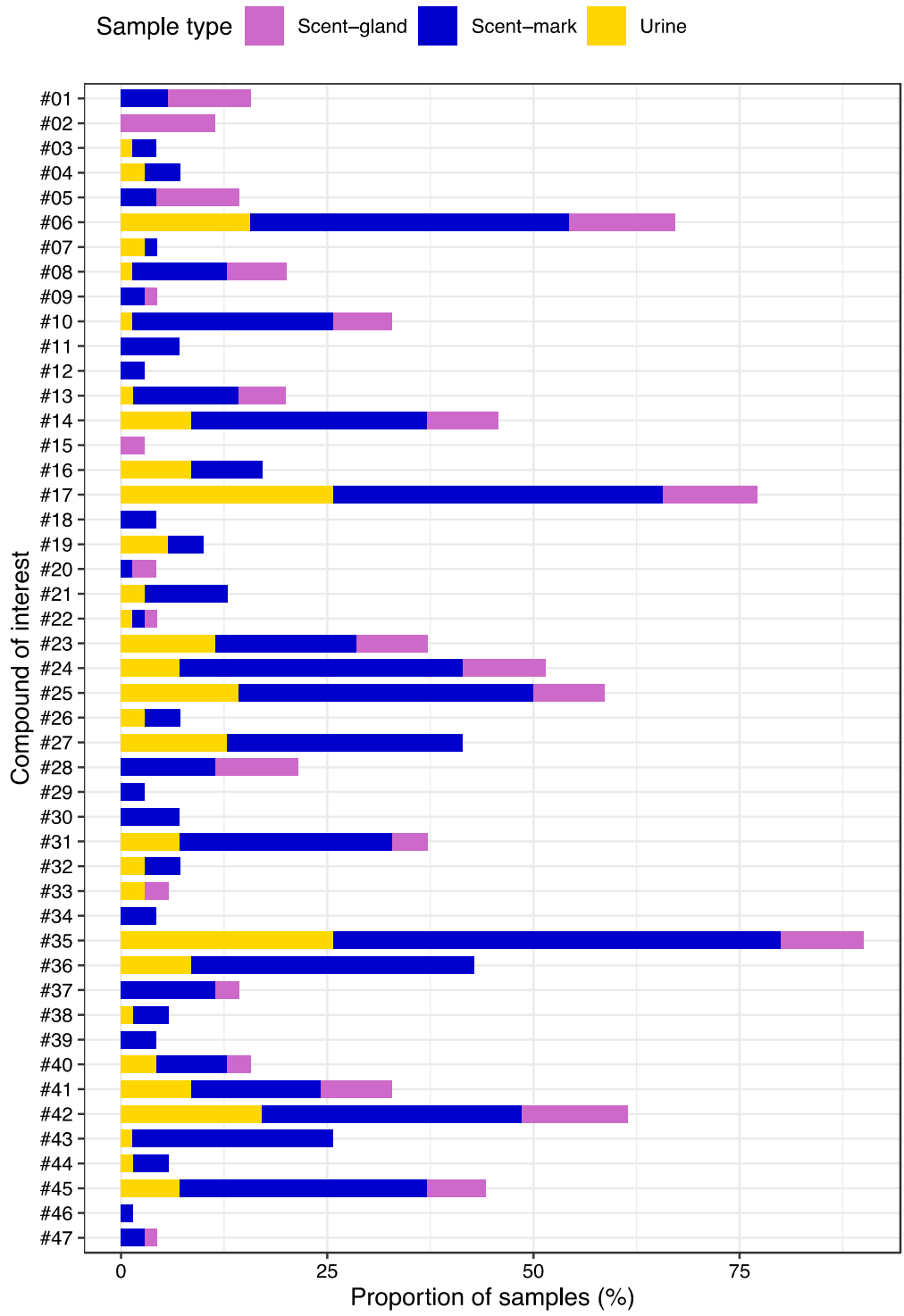


Figure III.15. Distribution of the 47 compounds of interest retrieved from the samples of the five primate species studied across scent-gland secretions, deposited scent-marks, and urine (animal samples dataset). Compounds' names are given in **Table III.7**.

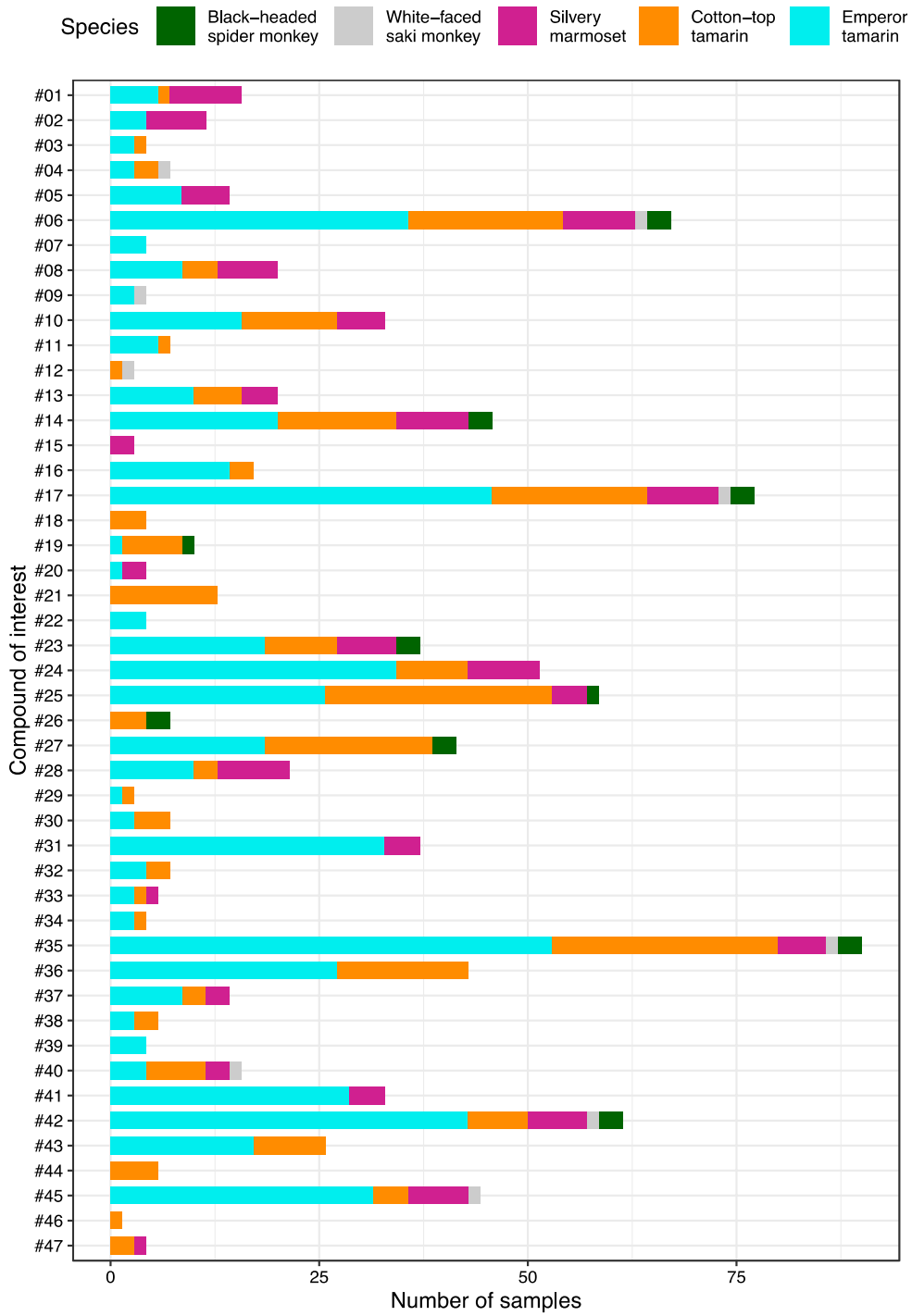


Figure III.16. Distribution of the 47 compounds of interest retrieved from the samples across the five primate species studied: black-headed spider monkey, *A. fusciceps*, silvery marmoset, *M. argentatus*, white-faced saki monkey, *P. pithecia*, emperor tamarin, *S. imperator*, and cotton-top tamarin, *S. oedipus* (animal samples dataset). Compounds' names are given in **Table III.7**.

III.3.3.2.2. Occurrence of compounds of interest in the different tamarin groups

Most compounds from the tamarin samples dataset (N=46) were common to all four tamarin groups (26.1%) or shared by the emperor tamarin group at TZ and the cotton-top tamarin group at PWP (26.1%; **Fig. III.17**). 6-methyl-5-hepten-2-one (#20) was only retrieved from samples from the emperor tamarin group at DMP; and 1,2-dimethylbenzene (#12) and tetra- or penta-decanal (#46) from the cotton-top tamarin group at DMP. Furthermore, 1,3,5-trimethylbenzene (#18), 1,2,3- or 1,2,4-trimethylbenzene (#21), p-cymene (#26), 2-methyldecylpropanoate (#44), and heptadecane (#47) were specific to the cotton-top tamarin group at PWP. Finally, 1-methoxypropan-2-ol (#02), propane-1,2-diol (#05), 2-methylpropanoic acid (#07), 3-methylbutanoic acid (#09), benzyl alcohol (#22), *trans*-1-methyl-4-(1-methylethyl)cyclohexanol (#31), 3-methyltridecane (#39), and diethylene glycol dibutyl ether (#41), were uniquely found in samples from the emperor tamarin group at TZ (**Fig. III.17**).

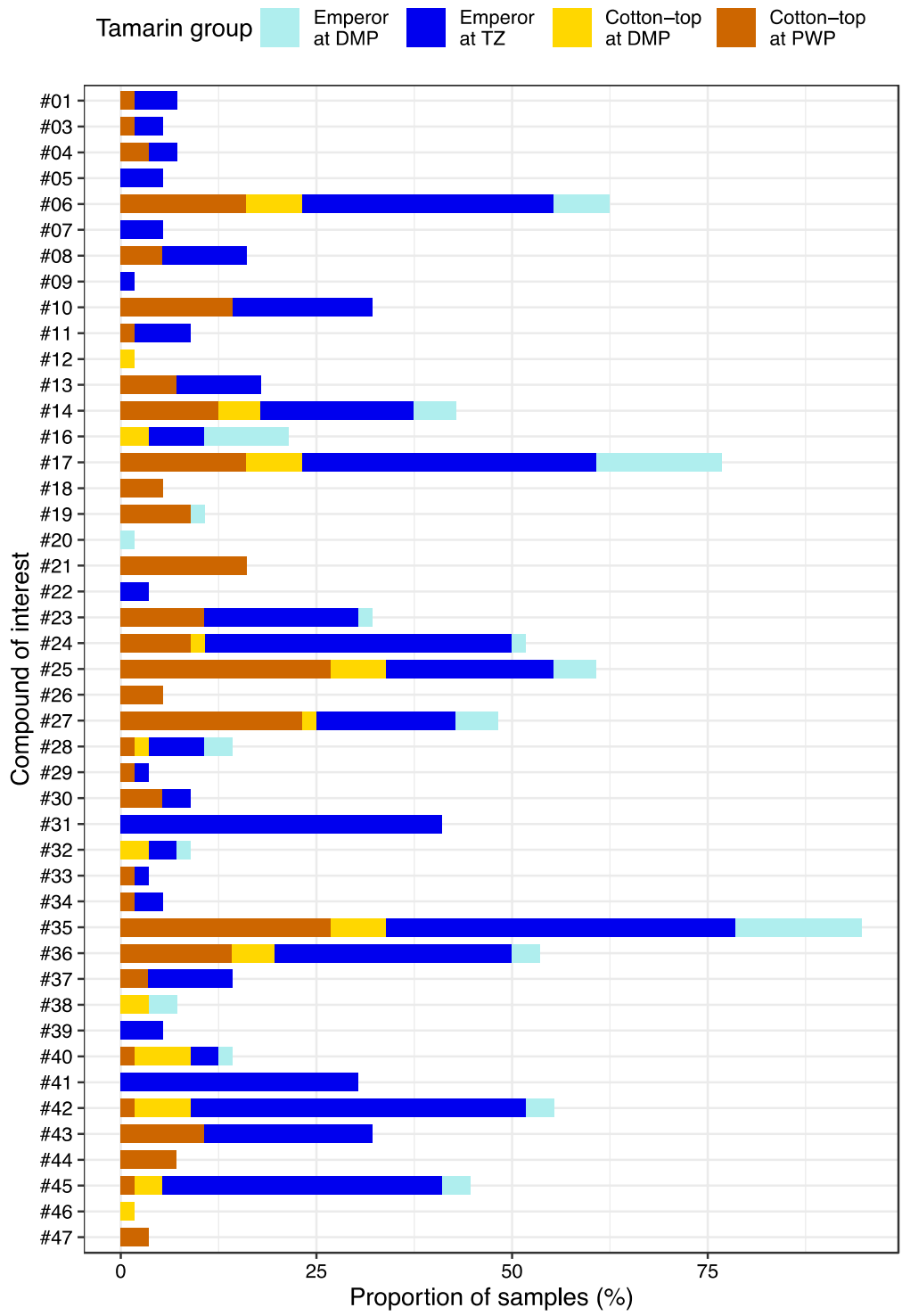


Figure III.17. Distribution of the 46 compounds of interest retrieved from the samples across the four groups of emperor and cotton-top tamarins (tamarin samples dataset; compound #15 absent from dataset). Compounds' names are given in **Table III.7**.

III.3.3.2.3. Occurrence of compounds of interest across sex, reproductive status, and individual female tamarins

The majority of compounds were shared between males and females (89.1%; **Fig. III.18**), and between reproductive and non-reproductive individuals (78.3%; **Fig. III.19**; tamarin samples dataset). Five compounds were uniquely found in females: 3-methylbutanoic acid (#09), 2-cyclopenten-1,4-dione (#11), 1,2-dimethylbenzene (#12), 6-methyl-5-hepten-2-one (#20), and tetra- or penta-decanal (#46); whereas no compound was specific to males (**Fig. III.18**). Similarly, eight compounds were only retrieved from samples from reproductive tamarins: 3-methylbutanoic acid (#09), 1,2-dimethylbenzene (#12), 6-methyl-5-hepten-2-one (#20), 1-nonanol (#32), the branched C8 carboxylic acid (#33), 3-methyltridecane (#39), tetra- or penta-decanal (#46), and heptadecane (#47). Only two compounds were only found in samples from non-reproductive tamarins: 1,3,5-trimethylbenzene (#18) and p-cymene (#26; **Fig. III.19**). Moreover, when comparing the six reproductive and non-reproductive adult female tamarins only, 6-methyl-5-hepten-2-one (#20) was unique to the reproductive female emperor tamarin at DMP; 1,2-dimethylbenzene (#12) and tetra- or penta-decanal (#46) were specific to the reproductive female cotton-top tamarin at DMP; and 3-methylbutanoic acid (#09) was only found in samples from the reproductive female emperor tamarin at TZ.

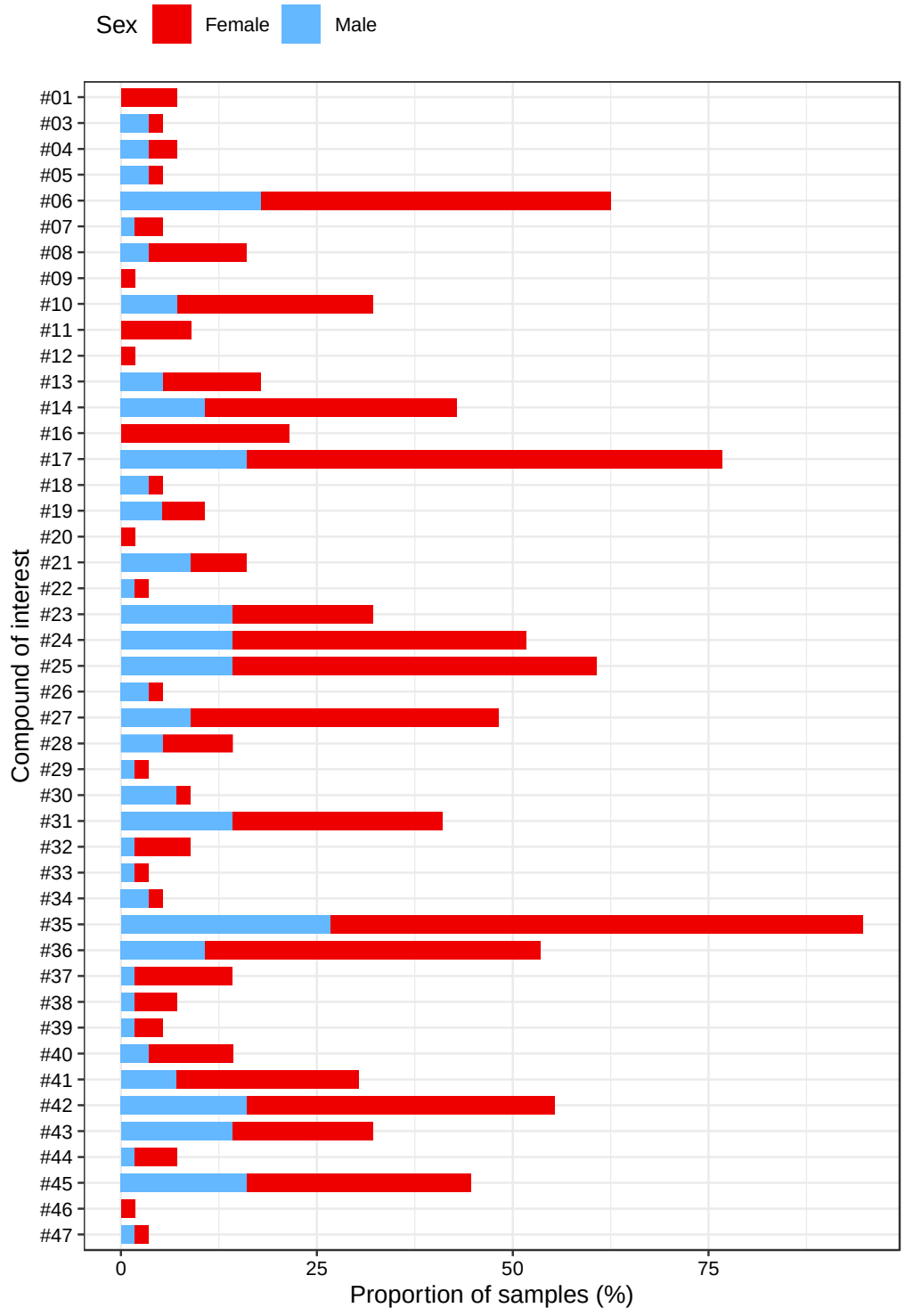


Figure III.18. Distribution of the 46 compounds of interest retrieved from the samples across males and females of the two tamarin species, emperor and cotton-top tamarins (tamarin samples dataset; compound #15 absent from dataset). Compounds' names are given in **Table III.7**.

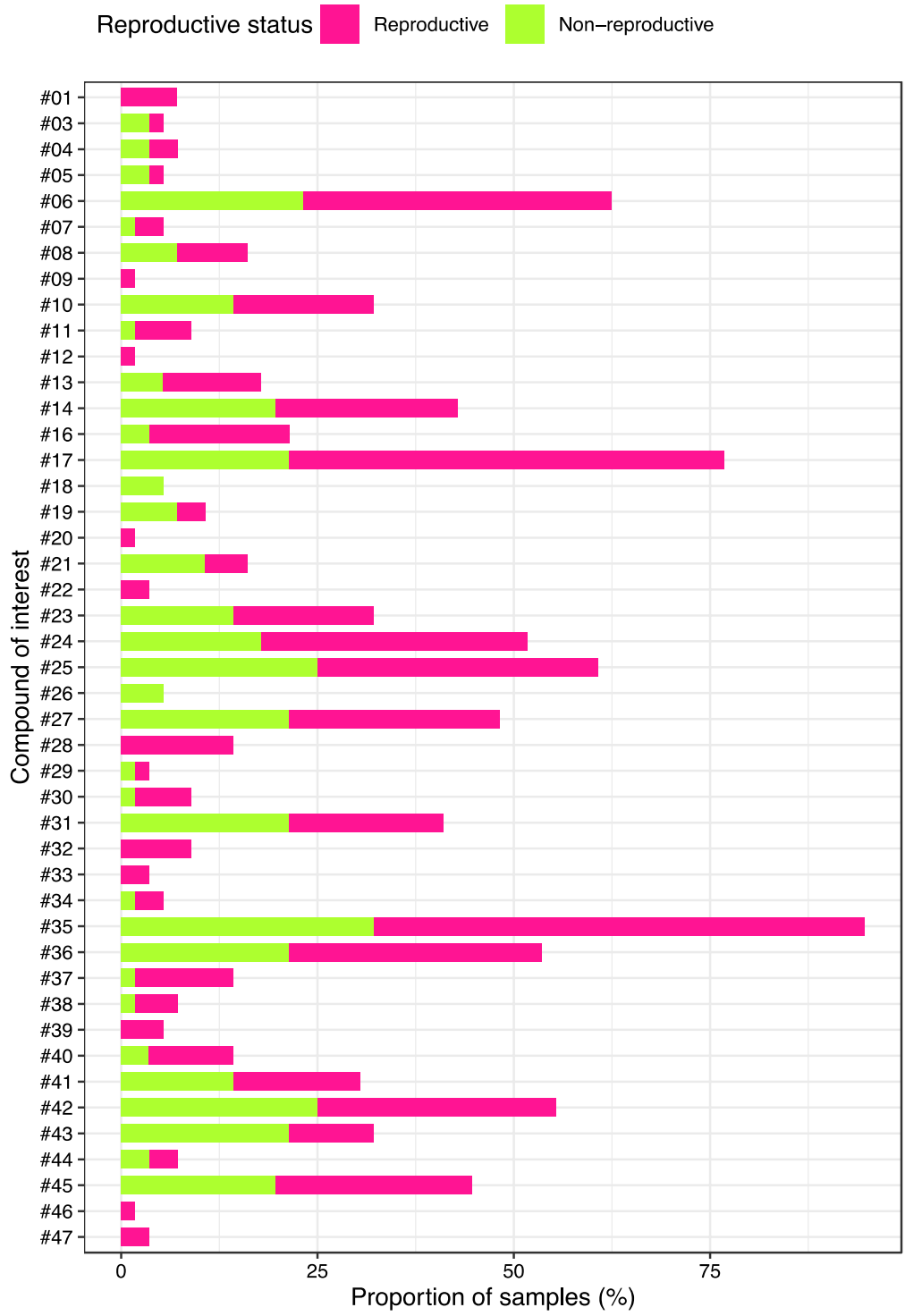


Figure III.19. Distribution of the 46 compounds of interest retrieved from the samples across reproductive and non-reproductive individuals of the two tamarin species, emperor and cotton-top tamarins (tamarin samples dataset; compound #15 absent from dataset). Compounds' names are given in **Table III.7**.

III.3.4. Experimental test of temporal stability of sample chemical composition

Temporal stability was tested using tamarin scent-mark samples of lower quality than the ones used in the previous section (see section III.2.2). The conditions experimentally tested were **1.** samples extracted five times with short delay between extractions (i.e. 1.5 hour); **2.** samples extracted five times with long delay between extractions (i.e. 10–24 hours); and **3.** samples extracted twice with long delay between extractions (i.e. 72 hours). In all three conditions, sample chemical richness decreased (i.e. compounds were lost) after the first extraction, and at each following extraction (**Fig. III.20**).

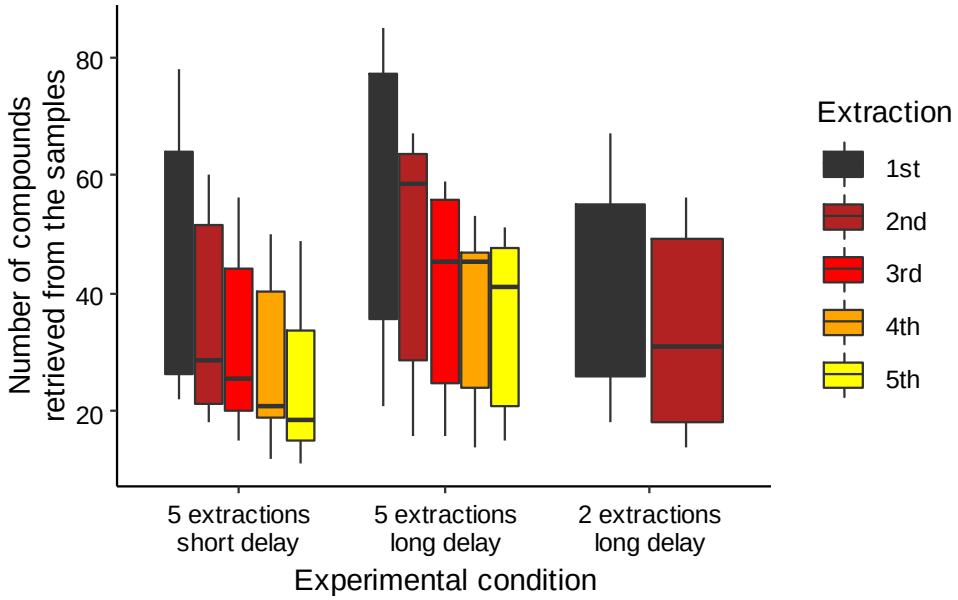


Figure III.20. Tukey's box-and-whiskers plot showing variation in the number of compounds (i.e. chemical richness) retrieved from samples tested under three experimental conditions: samples extracted five times at short (1.5 h), and long (10–24 h) interval, and samples extracted twice at long interval (72 h). Six samples were run under each experimental condition. Boxes indicate the median and interquartile range (IQR); whiskers give the smallest value ≥ lower hinge -1.5*IQR, and largest value ≤ upper hinge +1.5*IQR.

Kruskal-Wallis rank sum tests run on each of the three experimental conditions, followed by pairwise Dunn's post-hoc tests, indicated an absence of significant difference between repetitive extractions. Nevertheless, further results from a generalized linear mixed model showed

a significant effect of the number of extractions on sample chemical richness, even at the second extraction. However, no significant difference was found between experimental conditions, i.e. number of extractions and delay between extractions (**Table III.9**). These results indicate that repeated extraction of the same sample had the most effect on the diminution of compounds retrieved, regardless of whether the samples were left at room temperature for a short or long period between successive extractions.

Table III.9. Results of a generalized linear mixed model (*Poisson* family, log link function) testing the effect of experimental conditions, and the two or five successive extractions, on the number of compounds retrieved from the samples. *P*-values were significant at $P \leq 0.05$ (in bold). SE: standard error of the mean, SD: standard deviation.

Fixed effects	**Paired comparisons**	**Estimate (± SE)**	***Z*-value**	***P*-value**
(intercept)		3.687 (±0.213)	17.284	**< 0.001**
Condition	5 extractions short – 5 extractions long	0.263 (±0.298)	0.882	0.378
	5 extractions short – 2 extractions long	-0.056 (±0.302)	-0.186	0.853
Extraction	1_{st} – 2_{nd}	-0.228 (±0.051)	-4.508	**< 0.001**
	1_{st} – 3_{rd}	-0.361 (±0.061)	-5.935	**< 0.001**
	1_{st} – 4_{th}	-0.465 (±0.063)	-7.403	**< 0.001**
	1_{st} – 5_{th}	-0.542 (±0.064)	-8.414	**< 0.001**
Random effect		**Variance (±SD)**		
Sample ID		0.260 (±0.510)		

Looking solely at changes in sample chemical richness would fail to take into account the possible appearance with time of new compounds, owing to bacterial activity or other chemical reactions inside the sample vial at room temperature. Therefore, all compounds from the different treatments were subsequently separated into three categories: ***i.*** the lost compounds group, i.e. compounds present in the sample at first extraction, but absent at any of the following extractions; ***ii.*** the gained compounds group, i.e. compounds not present at first extraction and appearing at one

of the following extractions; and ***iii.*** the ambiguous compounds group, i.e. compounds retrieved at some extractions, but not others, from the same sample (e.g. a compound appearing at the second extraction, absent from the sample in the third and fourth extraction, and retrieved again at the fifth extraction). The ambiguous compounds group was removed from further analysis. **Fig. III.21** shows the cumulative proportion of compounds **a.** gained, and **b.** lost, accounting for the number of compounds retrieved from the samples at each extraction, in the three experimental conditions.

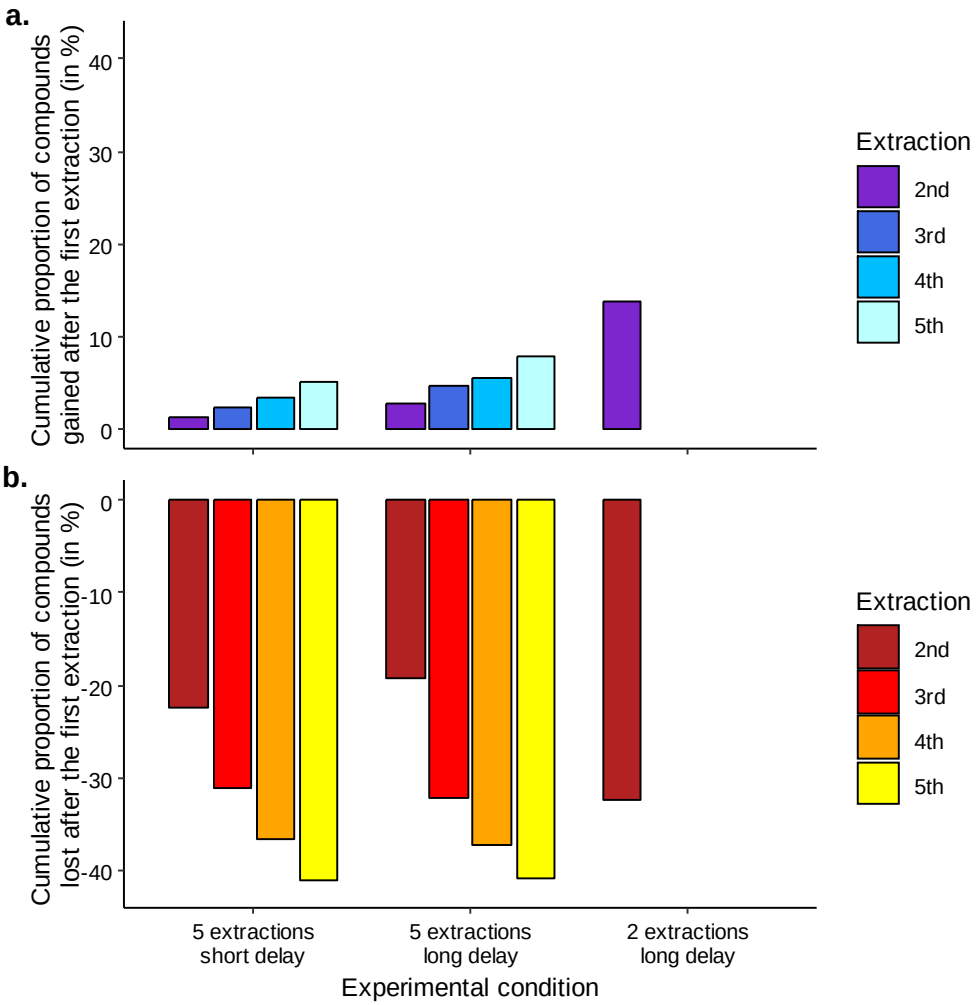


Figure III.21. Cumulative proportion (in %) of compounds **a.** gained, and **b.** lost, accounting for the number of compounds retrieved from the samples at each extraction for three experimental conditions: samples extracted five times at short (1.5 h), and long (10–24 h) interval, and samples extracted twice at long interval (72 h).

While up to 41% of compounds present at first extraction were lost over time, new compounds constituted up to 14% of the total compounds

retrieved from the samples. No difference was observed between the short- and long-delay conditions with five successive extractions. Measures of gained and lost compounds in the condition with only two extractions were very likely to be overrepresented, because it was not possible to distinguish between genuinely gained, or lost, compounds, and possible artefacts from the analyses (i.e. the ambiguous compounds category).

In addition, some of the compounds either having been lost, or having been gained between the first, and the second or third extraction, were tentatively identified using the NIST mass spectral library using a threshold of identity match of 80% and over, and visual comparison of the mass spectra (**Table III.10**).

Table III.10. Subset of the compounds lost and gained between the first, and the second or third extraction of the same samples. Tentative names were given by NIST mass spectral library search, with identity match ≥80%. SD= standard deviation.

Lost / Gained	Average retention time ±SD (min)	Compounds tentative name (identity match)
Lost	6.157 ±0.013	Hexanal (91%)
Gained	6.449 ±0.017	Butanoic acid (90%)
Gained	7.547 ±0.032	4-Hydroxypentan-2-one (83%)
Lost	8.996 ±0.002	o-Xylene (94%)
Lost	9.347 ±0.007	Heptanal (96%)
Lost	11.995 ±0.011	6-Methyl-5-hepten-2-one (93%)
Gained	12.131 ±0.053	Hexanoic acid (80%)
Lost	13.113 ±0.004	D-Limonene (99%)
Gained	13.471 ±0.010	3,3,5-Trimethylcyclohexanone (90%)
Gained	14.737 ±0.013	Heptanoic acid (94%)
Lost	14.884 ±0.006	2-Methoxyphenol (97%)
Gained	17.141 ±0.009	Octanoic acid (86%)
Lost	17.626 ±0.004	Methyl salicate (97%)
Gained	21.728 ±0.004	2,6,10-Trimethyldodecane

III.4. Discussion

III.4.1. Searching for chemical signatures in callitrichid scents

Results from the chemical analyses confirmed a difference in the chemical composition of scent-gland secretions, scent-marks, and urine, which are all used in olfactory communication, suggesting that they may each convey different scent messages, and/or serve different functions. Such variation in chemical composition is hardly surprising: upon deposition, glandular secretions would mix with the environment. In addition, bacterial activity and loss of the most volatile compounds potentially lead to a rapid change in the chemical composition of the scent-mark (Ezenwa and Williams, 2014), as inferred in the results of the experimental test of temporal stability of samples in section III.3.4. Results from this experiment will be discussed further in section III.4.3. Such variation with time and the effect of bacterial activity is also likely to happen for urine, as shown by Delbarco-Trillo et al. (2013), who found that voided urine from captive aye-ayes, *Daubentonia madagascariensis*, contained 17% more compounds than urine collected directly inside the animals' bladder. Moreover, scent-marks are often a mixture of secretions and urine, faecal materials and/or vaginal/seminal fluids (e.g. in wolves, *Canis lupus*, Asa et al., 1985; and giant pandas, *Ailuropoda melanoleuca,* Hagey and Macdonald, 2003), which could further explain the chemical differences observed with scent-gland secretions. Urine, the production of which involves a different biochemical pathway than glandular secretions, is believed to contain different, less volatile compounds (Müller-Schwarze, 2006). Nevertheless, the present results also established that many urine and glandular volatile compounds are shared, which has been reported across many mammalian taxa (e.g. reviews by Apps, Weldon and Kramer, 2015; and Charpentier et al., 2012; see **Table V.1** in **Chapter 5** for a comprehensive review of all compounds of interest identified in this study).

Statistical analyses further confirmed the existence of particular chemical signatures in the scent samples, at the levels of species and group. Other studies have found similar results in primates and other mammalian taxa, with differences between species (e.g. in glandular secretions of two owl monkeys, *Aotus azarae* and *A. nancymaae*, Spence-Aizenberg et al., 2018; urine of brown lemurs, DelBarco-Trillo et al., 2011;

anal gland secretions of sympatric Siberian weasels, *Mustela sibirica*, and steppe polecats, *M. eversmanni*, Zhang et al., 2002; swabs from cheeks and forehead of large felines, Soini et al., 2012; and urine of several *Phodopus* hamster species, Soini et al., 2005), subspecies (e.g. in the anal gland of Scandinavian beavers, *Castor fiber fiber* and *C. f. albicus*, Rosell and Steifetten, 2004), and groups (e.g. in axillary odours of rhesus macaques, *Macaca mulatta*, Weiß et al., 2018a; sternal gland secretions of mandrills, Vaglio et al., 2016; anal gland secretions of meerkats, Leclaire et al., 2017; and interaural gland secretions of Bechstein's bats, *Myotis bechsteinii*, Safi and Kerth, 2003). Nevertheless, species and group chemical signatures observed in this study may have been partly masked by differences between study sites, as a result of the unbalanced sampling design (i.e. not all species were present at all zoos). Although it was removed from the global linear models testing the effect of all factors on sample chemical richness and diversity, study site appeared to affect sample chemical diversity (section III.3.2.2.2). In a wild environment, differences between study sites would represent variation between distinct animal populations, which may be linked to different habitats, food resources, commensal bacteria communities, and genetic dissimilarities (e.g. variation in the chemical composition of anal gland secretions of geographically distinct wild populations of Eurasian otters in England and Wales, Kean et al., 2017; chin gland secretions of European rabbits, *Oryctolagus cuniculus*, across southeastern Australia, Hayes et al., 2002; and interaural secretions of several wild colonies of Bechstein's bats in Germany, Safi and Kerth, 2003). In captive conditions however, it may correspond instead to the husbandry under which captive primates are housed. Differences in diet, elements of the enclosure, husbandry procedures (e.g. enrichment routines), climate and illumination, etc., can play a role in the bacterial environment (Clayton et al., 2016), and therefore influence the compounds produced (e.g. the effect of diet on ring-tailed lemurs' genital secretions, Drea et al., 2013; and on the volatile compound composition of mouse urine, Kwak et al., 2008).

In addition, chemical differences were observed at the levels of sex, reproductive status, and between individual female tamarins. Sex differences have been reported in other primates (e.g. in genital secretions of Coquerel's sifakas, Greene and Drea, 2014; subcaudal secretions of owl monkeys, *A. nancymaae*, MacDonald et al., 2008; and sternal gland

secretions of mandrills, Setchell et al., 2010), and carnivores (e.g. in urine of lions, *Panthera leo*, Andersen and Vulpius, 1999; and binturongs, *Arctictis binturong*, Greene et al., 2016b; anogenital gland secretions of giant pandas, Hagey and Macdonald, 2003; Yuan et al., 2004, and anal gland secretions of banded mongooses, *Mungos mungo*, Jordan et al., 2011; brown bears, *Ursus arctos*, Rosell et al., 2011; and ferrets, *Mustela furo*, Zhang et al., 2005). Similarly, chemical signatures of reproductive state (e.g. in genital secretions of female Coquerel's sifakas, Greene and Drea, 2014), and dominance status (e.g. in sternal gland secretions of male mandrills, Setchell et al., 2010), concur with the present findings of an effect of callitrichid reproductive status on the chemical composition of their deposited scents. In addition, individual differences in chemical composition have previously been reported in a number of primates (e.g. in genital and brachial gland secretions of ring-tailed lemurs, Boulet et al., 2010; Charpentier, Boulet and Drea, 2008; Scordato, Dubay and Drea, 2007; sternal gland secretions of mandrills, Setchell et al., 2010; and deposited scent-marks of common marmosets, Smith et al., 2001b; Smith, 2006), as well as in other mammals (e.g. in sternal gland secretions of koalas, *Phascolarctos cinereus*, Salamon and Davies, 1998; subcaudal gland secretions of European badgers, *Meles meles*, Buesching, Waterhouse and Macdonald, 2002a; b; anogenital gland secretions of giant pandas, Hagey and Macdonald, 2003; anal gland secretions of spotted hyenas, *Crocuta crocuta*, Burgener et al., 2009; and ferrets, Zhang et al., 2005; and interaural gland secretions of Bechstein's bats, Safi and Kerth, 2003). Similar to the results presented on scent-marking behaviour in **Chapter 2**, section II.4.1.1, chemical differences at the levels of sex, reproductive status, and the individual, may indicate a role of chemosignalling in mate choice, intrasexual competition, dominance and/or reproductive suppression in callitrichids.

III.4.2. Identity of compounds of interest in callitrichid scent samples

The total number of compounds found in samples from the two tamarin species (N=281), for which most samples were collected, was in the range of previous findings in primate semiochemistry: Smith et al. (2001b) found 162 compounds in scent-marks of female common

marmosets; Greene and Drea (2014) detected 252 compounds in the genital secretions of Coquerel's sifakas; MacDonald et al. (2008) found 300 volatile compounds in the subcaudal gland secretions of owl monkeys; but Setchell et al. (2010) found only 47 compounds in 88 swabs of mandrill sternal gland secretions; and Delbarco-Trillo et al. (2011) retrieved 74 volatiles from the urine of twelve species of brown lemurs.

Many of the compounds identified in this study by mass spectral library search (N=47) have been reported in previous work on mammalian semiochemistry (see **Table V.1** in **Chapter 5**). In addition, I was able to verify the identity of 22 of these compounds, by comparison of retention times with those of commercially obtained compounds. In cases when the retention time of the compound from the biological sample, and that of the commercial compound, were too different for them to be the same compound, it was still possible to infer putative identity of the compound, if its mass spectrum was very similar to that of the commercially obtained compound. This was the case when compounds of similar mass spectra to that of their corresponding commercial compounds yet eluted earlier. For example, I inferred compound #33 to be a branched chain C8 carboxylic acid, as its mass spectrum was identical to that of commercial octanoic acid, but its retention time was significantly less. In a series of structural isomers, such as C8 carboxylic acids, the retention time reduces as the degree of branching increases, owing to reduced van der Waals' attraction between the molecule and the stationary phase in the GC-MS. Identified compounds of interest were mainly alcohols, hydrocarbons, aldehydes, and ketones, sometimes containing an aromatic group, which have been mentioned in reviews on mammalian semiochemicals (Apps, Weldon and Kramer, 2015; Charpentier et al., 2012).

A few of the identified compounds appeared to be specific to single sample categories. Notably, 2-methylpropanoic acid (#07) and 3-methyltridecane (#39) were unique to emperor tamarins; 1,3,5- and 1,2,4-trimethylbenzene (#18 and #21), 2-methyldecylpropanoate (#44), and tetra- or penta-decanal (#46) to cotton-top tamarins; and 2,5-dimethylpyrazine (#15) to silvery marmosets. Therefore, these compounds may play a role in species-specific chemosignalling. Moreover, 3-methylbutanoic acid (#09), 1,2-dimethylbenzene (#12), 6-methyl-5-hepten-2-one (#20), and tetra- or penta-decanal (#46), which were only retrieved from reproductive female

tamarin samples, might be indicators of female reproductive state, and hence constitute important cues for breeding and/or reproductive suppression in the genus *Saguinus*. Yet all of these compounds, except #39 and #44, have also been found in secretions and/or urine from other mammals (see **Table V.1** in **Chapter 5**). Nevertheless, the same compound may very well serve different functions in different species. For instance, Goodwin et al. (2006) revealed the existence of well-known bark beetle (Scolytinae) aggregation pheromones frontalin, exo-brevicomin, and endo-brevicomin, as well as the aphid (Aphidoidea) alarm pheromones (E,E)-α-farnesene and (E)-β-farnesene, in urinary chemical signals from ovulatory female African elephants, *Loxodonta africana*.

Importantly, several of the compounds of interest listed in **Table III.6** may have not been directly produced by the animals. For example, anisole (#16), p-cresol (#25), p-cymene (#26), 2-methoxyphenol (#27), and eugenol (#38), are definitely of non-mammalian origin, because their metabolic pathway only exists in plants, fungi and bacteria (Charpentier et al., 2012). Nevertheless, these compounds may originate from diet or commensal bacteria. A well-known example of the use of substances derived from alimentation in animal communication is that of feather colouration in birds (Endler, 1980). Birds acquire carotenoids from food, which once passed to the tegument of their feathers provide bright colouration. Plumage colouration has become an important honest signal of health, favoured by sexual selection. Similarly, Ferkin et al. (1997) experimentally demonstrated that differences in diet affected the attractiveness of meadow voles, *Microtus pennsylvaticus*, to the other sex. Moreover, commensal bacteria present in the scent-glands or on the skin may take an active part in the composition of the secretions, as seen in section III.4.1. Such findings were acknowledged in mammalian studies such as by Goodwin et al. (2012), on African elephants; Leclaire et al. (2014; 2017) on meerkats; and Theis et al. (2012; 2013) on spotted hyenas, and striped hyenas, *Hyaena hyaena*. Some compounds of interest given tentative identities in the present study were also found to be produced by human commensal bacteria using similar analytical methods (i.e. 2-methylpropanoic acid, 2-methylbutanoic acid, heptanal, benzaldehyde, and benzeneacetaldehyde found in the human faecal microbiome, Raman et al., 2013; and pentan-2-one retrieved from cultures of *Staphylococcus aureus*, Tait et al., 2014). In addition, some compounds

of plant origin may have been incorporated into the swabs during sampling and correspond to the odour of the substrate branches themselves, or to contamination of the surface swabbed by food remains and/or excrement. Nevertheless, callitrichids, as many animals, may very well use such extraneous compounds as chemosignals, even if they do not directly produce them. Archie and Theis (2011), and Ezenwa and Williams (2014), offer good reviews of the relationships between microbial communities and animal olfactory communication.

III.4.3. Temporal stability of sample composition

Results from the sample decay experiment confirmed the loss of over 40% of compounds in samples over time, and showed that repeated extraction of a sample enhanced the loss of compounds. Such results reveal the low concentration of many of the compounds retrieved from these samples, for them to be removed completely at first or second extraction using the SPME fibre. Moreover, samples not only lost compounds over time, they also gained new compounds, with over 5% of new compounds appearing over time after successive extractions. These new compounds may originate from the degradation of previous compounds at room temperature, and/or via bacterial activity inside the sample vial. When comparing some of the compounds lost from the samples with those newly gained, it appeared that some of the new compounds appeared to be a degraded version of the compounds of origin. For instance, while hexanal and heptanal disappeared at the second extraction, hexanoic acid and heptanoic acid appeared. Hexanal and heptanal may well have been oxidized into their corresponding carboxylic acids via action of microorganisms inside the vial when left at room temperature. Other compounds, such as 2,6,10-trimethyldodecane, are known products of bacteria chemical pathways. Besides, some compounds, such as 2-methoxyphenol, are quite reactive when exposed to air and light, and would have naturally degraded when left at room temperature. Such natural decay of deposited scent-marks and urine may in fact participate to the information conveyed in chemosignals, as the progressive loss and/or replacement of compounds in an odour would relate to the time elapsed since the signaller animal was physically present

in the area, and/or displaying a certain physiological state such as ovulation (Goodwin et al., 2012; Müller-Schwarze, 2006).

Finally, the results showed no evidence that long delays at room temperature, as opposed to short delays, affected more sample chemical composition, which implies that temperature may not directly impact sample quality to a damaging level. Nevertheless, the variability in chemical composition observed over time in the samples used for this experiment, analysed with SPME–GC-MS, supports the recommendation made by many authors in the field of mammalian semiochemistry to keep samples frozen until their analysis, to avoid instability as much as possible (e.g. Apps, Weldon and Kramer, 2015; Drea et al., 2013).

III.4.4. Limitations of the study

The present study showed evidence for various types of chemical signatures in callitrichid scents, and tentatively identified 47 compounds as putative semiochemicals. Nevertheless, the chemical sampling and analysis procedure presented several flaws. First, the sample size was limited to one or two groups of each callitrichid species, which restricted the interpretation of the present chemical results. In addition, the two groups of emperor and cotton-top tamarins were studied at three different sites (i.e. one group of emperor tamarin at TZ, one group of cotton-top tamarin at PWP, and one group of each at DMP), which did not permit the strict distinction between site, species, and group differences in the linear model, nor in the permutational analysis of variance. Besides, collection of suprapubic scent-gland swabs only for silvery marmosets and emperor tamarins at TZ did not allow comparisons with other species or sites. Additionally, samples from silvery marmosets were unfortunately very scarce (i.e. only one suprapubic gland swab was collected per individual during health check), thus preventing multiple comparisons with other species or sample types to be made. Finally, it was not possible to collect multiple samples from all animals included in this study, owing to limited access into the enclosures and constraints in the amount of time dedicated to sample collection overall. Moreover in some instances, while collection of repeat samples was achieved, some did not pass the sample quality assessment and had to be removed from the pool of samples used in the

analyses. The total sample size for this project would have preferably included at least two repeats of all three sample types (i.e. scent-gland secretion, deposited scent-mark and urine) for each individual in all the groups studied, and at least one occurrence of two groups of the same species hosted at the same zoo. However, such conditions were not possible to follow in the present study (see **Chapter 2**, section II.4.2).

Although scent-marks were collected as fresh as possible, access into the enclosure was not always readily possible, thus affecting the quality of samples. Moreover, in a zoo environment enclosure elements used as scent-marking substrates, such as dry branches and platforms, are at places covered by several layers of old food remains, faeces, scent-marks and urine, potentially strongly affecting the smell swabbed after a scent-marking event. Similarly, the ambient air in a captive tamarin enclosure, which is a small enclosed space, is saturated with odours of food, faeces, urine, and other body odours, forming a strong background smell. In wild conditions, the use of a wider range of substrates, and regular rainfall, might ensure a more neutral substrate prior to scent-marking. The present study was initially designed to use branch swabs (i.e. swabs of branch substrate on a spot not covered with a freshly deposited scent-mark), and air swabs (i.e. swabs held in the enclosure without touching anything) as controls, to differentiate urine and scent-mark volatile compounds from the enclosure's 'chemical background'. The chemical results showed that although branch and air compounds could be distinguished from those of scent-marks and urine via non-metric multidimensional scaling (see section III.3.2.2.1), their chemical composition was quite similar. The original data processing methodology was to remove all compounds found in branch or air samples as well as in the genuine animal samples. However, many of the compounds retrieved from branch and air samples might in fact have originated from scent-marks or urine deposition, in such a closed environment. It was thus decided to include these compounds, provided that their tentative NIST identity or mass spectrum was not that of obvious contaminants. There is therefore no confident way in the present dataset, to tell apart genuine compounds from a scent-mark freshly collected, from compounds already present in the air or on substrates at the time of deposition, because these can in fact be the same compounds. Nevertheless, the analytical techniques employed in the present study (i.e. SPME–CG-MS) were designed to retrieve only the most volatile

compounds from the swabs; hence, they increased the likelihood that the compounds identified from the animal swabs were newly produced instead of having been present prior to deposition, or else they would have evaporated by the time of swab collection.

Another limitation of the sampling procedure was that it did not allow for the control of the amount of sample collected on each swab, making impossible any attempt at measuring individual concentration of the different compounds. Even though a standardized swabbing, where the swab was rubbed for the same number of times and in the same fashion for each sample, was employed, there was an important variation in the amount of secretion or urine collected each time (pers. obs.). In addition, the mass spectrometric detector has different sensitivities for different compounds. This means that whilst the area of a peak on a chromatogram is proportional to the concentration of the corresponding compound in the sample injected, peak areas of different compounds are not proportional to their relative concentrations. Nevertheless, it is likely that the amount of scent-gland secretion deposited in a scent-mark at one time constitutes an aspect of the chemical message broadcast by the signalling individual (Müller-Schwarze, 2006). Callitrichids may be more sensitive to certain compounds than others, and to their relative concentration as well as their identity, as shown by Laska and co-workers on other primates (Laska et al., 2004; Laska, Wieser and Hernandez Salazar, 2005; Laska et al., 2007; Eliasson, Hernandez Salazar and Laska, 2015). Hence unfortunately the chemical messages exchanged by these animals have the potential to be much more complex than the level of analysis reached in this study.

In addition, it is essential to note that the sample extraction technique employed, headspace SPME, was selective for the more volatile components of the samples (see **Chapter 1**, section I.2.2.2.3). Indeed, the compound of highest molecular weight identified in the present study was heptadecane, a C17 molecule (i.e. composed of 17 carbon atoms), while other studies using different methods retrieved compounds up to C24 or C26 (e.g. Zhang et al., 2008, on the giant panda). The true ‘odour bouquet’ conveyed in individual callitrichid scent-marks is likely to be more complex than the assemblage of compounds retrieved from the samples in this study.

Finally, despite my best intentions to maintain the analytical equipment at the same level of precision throughout the project, some variation in the quality of the analyses happened. For example, the GC-MS instrument used for this project was serviced between the analysis of samples from TZ and PWP, and that of samples from DMP. GC-MS users would acknowledge that any variation in the components or parameters of the instrument may lead to a change in the analytical results, which are very difficult to identify. Besides, the five SPME fibres used throughout the project to extract volatile compounds from the samples' headspace, were at different stages of ageing for each sample. This potentially influenced the number of chemicals retrieved, as fibres lose efficiency as they age, and the amount of contamination found, as contamination on the fibre tends to increase the more it is used.

III.4.5. Conclusion

In spite of its limitations, the present study sheds light on the chemical composition of scent-gland secretions, scent-marks and urine deposited by callitrichids in a captive environment. Whatever messages are ultimately encoded in the glandular secretions produced by the animals, it is important to remember that they are naturally altered as soon as they are deposited, by mixing with other body fluids such as urine, and with chemicals from conspecifics, plants and microorganisms present in the environment, and due to post-deposition odour decay. The resulting chemosignals transmitted to con- and hetero-specifics hence reach a great level of complexity that will be difficult to decipher, even with the progress of analytical techniques. As is recognized in the field of mammalian semiochemistry (Apps, 2013; Drea et al., 2013; Müller-Schwarze, 2006; Wyatt, 2014a), combining chemical analyses with behavioural bioassays may be a good way to reach further into understanding the variable function of the diverse forms of chemical messages used in olfactory communication.

Chapter IV – Scent-gland semiochemistry in two wild sympatric tamarins

Abstract

In this chapter, I presented results from the first use in the field of the Torion® T-9 portable GC-MS device for *in situ* analysis of wild mammal scent samples, and compared them with similar results using a conventional bench-top GC-MS in the laboratory after transporting the samples back to the UK. Scent-gland and skin swabs were collected from 15 groups of two sympatric species of wild emperor tamarins, *Saguinus imperator*, and Weddell's saddleback tamarins, *Leontocebus weddelli*, during two successive seasons of a long-term capture-and-release programme in the Peruvian Amazon. I found chemical signatures in the samples at the levels of species, group, sex, reproductive status, the individual, and between sample types (i.e. anogenital, suprapubic, and sternal gland, and the skin of the inner thigh or arm). Moreover, I tentatively determined the identity of putative tamarin semiochemicals for 11 compounds of interest retrieved from *in situ* analyses, and 25 compounds from laboratory analyses. The use of the Torion® GC-MS yielded results of lower quality than the laboratory analyses; I provided recommendations for future use of this instrument for *in situ* analyses of mammalian chemical signals. In addition, I compared the chemical results obtained in the present field study with similar analyses in captive conditions.

IV.1. Introduction and hypotheses

IV.1.1. Challenges of semiochemistry studies in the wild

Knowledge of primate chemical communication is still limited (Heymann, 2006b), notably due to the methodological difficulties of recording and quantifying odour signals, especially in wild conditions (Drea et al., 2013). Nevertheless, this field of research has recently been building

up at a much faster pace, as mentioned in **Chapter 1**, section I.3. The development of modern analytical chemistry techniques has improved our ability to investigate a wider range of semiochemicals (i.e. of various molecular weight, polarity, or chemical class) from the scent-gland secretions and body odours of numerous mammalian species (see reviews by Apps, Weldon and Kramer, 2015; Burger, 2005; and Soso et al., 2014). However, state-of-the-art semiochemistry techniques do not resolve the common challenges faced by field biologists when studying animals in the wild, which are the collection, storage, and transportation of samples. As the chemical composition of scent samples is likely to change if they are not kept frozen (see **Chapter 3**, sections III.3.4 and III.4.3), the logistics of bringing samples from the field back to the laboratory can be difficult (Drea et al., 2013). At present, two approaches are used in the field of semiochemistry in wild conditions. The first approach is to collect samples on swabs (e.g. Leclaire et al., 2017; Weiß et al., 2018a) or sometimes, in the case of volatile compounds only, in thermal desorption tubes (e.g. Kücklich et al., 2017; Weiß et al., 2018b), and transport them to the laboratory for analysis by chromatography (see **Chapter 1**, section I.2.2). The second approach is to extract and analyse semiochemicals *in situ* using portable devices such as an electronic nose, which is able to identify an odour using the response pattern of an array of gas sensors to match a known odour pattern (Nagle, Schiffman and Gutierrez-Osuna, 1998). Electronic noses are notably used in human clinical research, such as for the screening of diseases in body odour and breath (reviewed in Röck, Barsan and Weimar, 2008). More recently, several chromatography companies have begun developing miniaturized gas chromatography-mass spectrometry (GC-MS) devices, such as the Torion® T-9 GC-MS developed by PerkinElmer (2016), which can conduct full chemical analysis of volatile components, while being portable. These devices were originally developed for the screening of specific volatiles in the fields of environmental science, food manufacturing, and chemical warfare, but are now starting to be used in the field of animal semiochemistry (e.g. analysis of the body odour of captive common marmosets, *Callithrix jacchus*, Kücklich et al., 2017). While promising, the use of these portable devices for the identification of a multitude of unknown mammalian semiochemicals is considerably more complex than screening for a few known compounds, and hence constitutes a further challenge.

IV.1.2. Aims and hypotheses

This chapter presents results from the chemical analysis of scent-gland and skin samples collected from two sympatric species of wild tamarins, analysed *in situ* using the Torion® T-9 portable GC-MS (PerkinElmer, 2016), as well as in the laboratory using a conventional bench-top GC-MS after transporting the samples back to Anglia Ruskin University (ARU). First, I investigated the existence of chemical signatures in wild tamarin scent-gland secretions at the levels of species, group, sex, and reproductive status, and between scent-gland types, in a similar approach as carried out in **Chapter 3** on captive callitrichid semiochemistry. Species, group and sex are markers of identity, likely to be conveyed in scent-marks in the form of chemical signatures (e.g. species differences in owl monkeys, *Aotus* spp., Spence-Aizenberg et al., 2018; group and sex differences in mandrills, *Mandrillus sphinx*, Setchell et al., 2010; Vaglio et al., 2016). Since chemosignalling is assumed to be involved in female primates' advertisement of reproductive status (Boulet, Charpentier and Drea, 2009; Semple and Higham, 2013; Snowdon et al., 2006), we can anticipate that breeding females might show a different, potentially more complex, range of chemicals in their scent-glands, used to advertise their current reproductive status. In addition, chemical signals may play a role in intrasexual competition in both males and females, for dominance and access to mates. Furthermore, the three tamarin specialized scent-glands (i.e. anogenital, suprapubic, and sternal glands) may also contain different semiochemicals, allowing different signals to be conveyed via one or another of the scent-glands, as suggested in ring-tailed lemurs, *Lemur catta* (Greene et al., 2016a).

Second, I examined the putative identity of the compounds retrieved from these wild samples using both *in situ* and laboratory-based approaches, which I compared with results from previous research on mammalian semiochemistry, including the compounds identified in **Chapter 3** on captive callitrichid semiochemistry. We can expect some of the compounds to be ubiquitous, found in all the samples analysed in this study as well as in other mammals; and others to constitute a unique signature of the species studies here (Wyatt, 2014a; see **Chapter 3**). I then discussed the success of the use of the Torion® GC-MS for the analysis of primate semiochemicals overall, as opposed to the widely used approach of

collecting samples in the field and transporting them to the laboratory for analyses. Finally, I provided advice for future field research using this device. I hope that the current study, though preliminary, will help decipher some of the signals conveyed in wild tamarin scents. Moreover, this study is aimed at developing novel techniques for the analysis of primate semiochemicals, in an effort to contribute to knowledge in the field.

IV.2. Methods

IV.2.1. Study site and species

Two sympatric tamarin species, the bearded emperor tamarin, *Saguinus imperator subgrisescens* (Hershkovitz, 1979, further referred to as *S. imperator*; **Fig. IV.1a**) and the Weddell's saddleback tamarin, *Leontocebus weddelli* (formerly *Saguinus fuscicollis weddelli*, Deville 1849, recently reassigned; Buckner, Alfaro, Rylands, & Alfaro, 2015; Matauschek, Roos and Heymann, 2011; **Fig. IV.1b**), were studied in June 2017 at Estación Biológica Los Amigos (EBLA) in the south-eastern Peruvian Amazon (12°34'S, 70°05'W).

Figure IV.1. Adults of the two study species: **a.** bearded emperor tamarin, *Saguinus imperator*, and **b.** Weddell's saddleback tamarin, *Leontocebus weddelli*. Photos credit: (a) S. Cassalett Malagón and (b) M. Guerra Vargas

EBLA is located in a 1400 km² conservation concession privately managed by the Peruvian non-governmental organization Asociación para la Conservación de la Cuenca Amazónica, under the umbrella of the international organization Amazon Conservation Association (**Fig. IV.2**).

Figure IV.2. Map of the location of the study site Estación Biológica Los Amigos (labelled Los Amigos on the map) in south-eastern Peru (12°34'S, 70°05'W). From the Amazon Conservation Association website (www.amazonconservation.org).

EBLA has an extended trail system incorporating a range of rainforest habitats, from extended palm swamps to high *terra firme* forest, able to host a great diversity and abundance of flora and fauna (Pacheco et al., 2009; Pitman et al., 2001). This richness in habitats and species, in

addition to relatively easy access (i.e. a 6–8 hours journey by car and motorboat from Puerto Maldonado), have made EBLA an attractive field site for a range of international research projects since its creation in 2001. Notably, the substantial primate community present at this site (i.e. 11 species) makes EBLA a place of choice for primatological studies (e.g. Pacheco et al., 2009; Palminteri, Powell and Peres, 2011; Watsa, 2013). At EBLA, saddleback tamarins are commonly found in groups of 3–9 individuals, with a group density of 2.0 groups per km², while emperor tamarins are found in groups of 2–7 individuals, with a group density of 1.5 groups per km² (Watsa, 2013). The two species often form mixed-species foraging troops during the day. Groups of both species are also observed travelling and associating with dusky titi monkeys, *Callicebus brunneus*, Goeldi's monkeys, *Callimico goeldii*, and Bolivian squirrel monkeys, *Saimiri boliviensis*, although less frequently (Pacheco et al., 2009; Palminteri, Powell and Peres, 2011; Watsa, 2013).

IV.2.2. Capture-and-release programme

Field Projects International (FPI, formerly Primates Peru), a non-profit organization based in Saint Louis, MO, USA, has been leading since 2009 one of the most remarkable long-term research projects at EBLA. FPI's research focuses on the demography, feeding ecology, social behaviour, communication, and parasites of emperor and saddleback tamarins (www.fieldprojects.org). The project involves an annual capture-and-release of 14–17 groups of tamarins of both species. Annual capture of the EBLA tamarin population enables individual growth and reproduction to be monitored, collection of an array of biological samples, and individual marking of all the animals using radio-collars, beaded collars, and tail bleach patterns (Watsa et al., 2015). FPI's capture-and-release programme is unique in repeatedly targeting multiple groups of two sympatric species of callitrichids in a relatively short time frame (i.e. 20–30 days of capture each year).

Tamarin groups are captured using baited mesh traps composed of six to eight compartments, individually fitted with a manually controlled door. Upon trapping, animals are sedated and processed singly for biological sampling and collar fitting, using a two-step sedation procedure

(Watsa et al., 2015). FPI's capture-and-release programme provided a great opportunity to obtain quick and non-invasive swabs of tamarin scent-glands while the animals were anesthetized. FPI's research is conducted with annual authorization from the Peruvian Ministry of the Environment (SERFOR), as well as the Animal Care and Use Committees of Washington University in Saint Louis, the University of Missouri–Saint Louis, and the University of Calgary (ACC protocol # AC15-0161). It adheres to the American Society of Primatologists' Principles for the Ethical Treatment of Non-Human Primates, and follows the Animal Behavior Society Guidelines (2018) and the American Society of Mammalogists' Guidelines on wild mammals in research (Sikes and Gannon, 2011).

IV.2.3. Scent-gland sample collection, extraction, and GC-MS analyses

For the purpose of this project, samples of tamarin scent-gland secretions were collected over 20 days in June 2017, during FPI's annual capture-and-release season, and analysed *in situ*. Sampling procedure consisted of gently wiping a sterile cotton bud, previously wetted with clean distilled water, over the scent-gland area of the skin. For each of the 29 emperor tamarins and 33 saddleback tamarins captured, the following swab samples were collected: **a.** anogenital scent-gland, **b.** suprapubic scent-gland, **c.** sternal scent-gland (or sternal skin area when not visible), and **d.** the animal's inner thigh, a relatively hairless region. In addition, **e.** two air controls (i.e. swab left out in the open for 30 sec, to control for background volatiles in the ambient air), and **f.** two blank controls (i.e. empty vial and unused swab) were collected per capture session. An entire tamarin group was captured at each successful trapping session, giving us the opportunity to collect samples from all animals in the group during the first phase of the sedation procedure. This permitted all swabs to be collected within a short time interval, and before the animals' scent-glands risked getting in contact with other surfaces, which would have introduced extraneous compounds in the swabs. Swabs were stored in new 4 mL glass screw-top vials fitted with a polytetrafluoroethylene/rubber septum (Supelco) and kept in a cool Thermos® flask filled with ice packs for a maximum of seven hours. Upon return from each capture session, samples were stored in a cold refrigerator at the field station until analysed (mean

temperature 0.5 ±2.4°C, recorded hourly by an automatized temperature data logger).

Scent samples were analysed at the field station an average of 3.8 ±2.8 days after collection, using headspace extraction and a new generation portable GC-MS (Torion® T-9; PerkinElmer, 2016) provided by Dr Amanda Melin at the University of Calgary (www.amandamelin.com). The samples were extracted individually using a 65 µm solid-phase microextraction (SPME) fibre coated with divinylbenzene/ polydimethylsiloxane (Custodion®; PerkinElmer, 2016) for 2 min at room temperature (23.9 ±3.1°C), after heating them for 2 min in a bath of simmering water (ca. 100°C). The volatile-coated fibre was then desorbed at 300°C for 5 sec into the injection port of the Torion® GC, which was equipped with a low polarity MXT-5 Low Thermal Mass capillary column. Helium was used as the carrier gas and the injection was on splitless mode, with split mode applied at 2 sec with a ratio of 10:1, then 50:1 at 10 sec. The temperature of the column started at 50°C, held for 10 sec, followed by an increase of 2°C/sec to the final temperature of 300°C, which was held for 2 sec. The total run time was of 137 sec per sample. Mass separation was performed by the Torion® toroidal ion trap MS under electron ionization mode at 70 eV, in full-scan mode in 45–500 mass-to-charge ratio (m/z) range. Each day, animal samples, blank and air controls, and fibre blanks (i.e. clean fibre run in the same conditions to control for carry over), were run in random order. This prevented batch effect, which is an artificial source of variation added to a group of samples during handling, equilibration, extraction, or GC-MS analysis. Although the Torion® possesses a built-in data interpretation software, all resulting chromatograms were taken back to ARU for further interpretation, because the Torion® was being used for another project after sample analysis.

An additional 29 scent-gland samples, collected during FPI's 2018 capture-and-release season, were shipped to ARU, where they were analysed by SPME–GC-MS following the same method as detailed in **Chapter 3**, section III.2.2. Temperature varied between below zero and room temperature during transportation (owing to unforeseen logistical difficulties, sample shipment between Peru and the UK was executed in several steps, during some of which the samples were left to thaw). These samples were collected from the anogenital, suprapubic and sternal scent-

glands, and a control region on the skin of the inner arm, of three males and five females belonging to two groups of emperor tamarins which had not been sampled during the 2017 season. Sample collection was performed following the same approach as detailed above, with the exception that viscose swabs held by forceps (the same as used in the captive study, see **Chapter 3**, section III.2.2) were employed instead of wet cotton buds.

IV.2.4. Interpretation of analytical results

For each GC-MS chromatogram, automatic peak detection, integration, and tentative identification using the National Institute of Standards and Technology (NIST) mass spectral library (Shen et al., 2014), was performed in ChemStation™ (Agilent, Santa Clara, CA, USA). All detected peaks were listed using the information of retention time, peak area and height, mass spectrum, and putative NIST identification. Only peaks with a minimum height of 1% of that of the largest peak were selected, in order to limit the inclusion of background noise. Additionally, in chromatograms generated *in situ* with the Torion® GC-MS, only peaks eluted before 1.6 min were selected, since peaks after this time generally displayed excessively noisy baselines (i.e. greater than one sixth of the highest peak); and peaks that were too flat to be distinguished from the baseline or from a neighbouring peak were removed from further analysis. Visual comparison of mass spectra between compounds originating from the tamarin scent-gland samples, the air and blank control samples, and the fibre blanks (i.e. when the clean SPME fibre was run like a sample), allowed the tentative identification of candidate tamarin semiochemical compounds, in the same way as in **Chapter 3** (section III.2.3).

IV.2.5. Determination of reproductive status

Reproductive status of all individuals of the two study species, defined as primary breeders, secondary breeders, and nonbreeders, was determined using a multivariate statistical algorithm developed by Watsa, Erkenswick and Robakis (2017). The model used indices of reproductive morphology (i.e. vulvar index, testicular volume, suprapubic gland area,

and mean nipple length), combined with known reproductive status of a subset of animals in the population obtained by direct observation of copulations, to predict the reproductive status of all other individuals (Watsa, Erkenswick and Robakis, 2017). Primary breeders were reproductively active males and females, regardless of whether they sired offspring. Secondary breeders were both males and females aged one to two years old and sexually mature, but not having bred yet. Nonbreeders were all offspring born that year and sexually immature.

IV.2.6. Statistical analyses

All statistical analyses were performed in R v.3.5.1 operated in RStudio (R Core Team, 2018). The limited number of samples collected, and of compounds retrieved from both *in situ* and laboratory chemical analyses, did not allow for the use of multivariate analyses to examine differences in chemical composition across samples, as was carried out in **Chapter 3**; therefore, most results in this chapter are solely descriptive. Nevertheless, in the case of the samples analysed *in situ* it was possible to test the influence of the factors species, group, sex, reproductive status, and sample type, on the likelihood of finding a compound of interest in the samples (i.e. compounds presence/absence). First, non-parametric Kruskal-Wallis rank sum tests (function kruskal.test() in R base package 'stats') tested variation in the presence/absence of compounds across the different factors. Dunn's tests with Bonferroni adjustment (function posthoc.kruskal.dunn.test() in R package 'PMCMR'; Pohlert, 2014) were then used as post-hoc tests to assess pairwise differences within the same factors. In the case of the samples analysed in the laboratory, Pearson's χ^2 tests with Yates' continuity correction (chisq.test() in base R package 'stats') were used to assess variation in the occurrence of compounds of interest across categories of group, sex, reproductive status, and sample type.

IV.3. Results

IV.3.1. *In situ* analyses of wild tamarin samples using the Torion® GC-MS

IV.3.1.1. Chemical differences at the levels of species, group, sex, reproductive status, and the individual, and between scent-glands

Swabs from the scent-glands and bodies of 62 animals in 14 tamarin groups of both species, as well as the ambient air, and blanks, were collected. Swabs presenting obvious contamination such as visible faecal material were excluded from the analysis. All samples from one emperor tamarin group (five animals) were excluded, because the GC-MS method used was refined after analysing these samples (e.g. change in run time, column temperature, injection conditions, etc.), which made the results non-comparable with the other samples. A total of 278 samples were hence included in the final analysis (**Table IV.1**; see **Appendix D**, **Table D.1**).

Table IV.1. Sample composition used for the *in situ* analysis of wild tamarin scent-gland secretion samples.

Species	# Groups	Sex	# Indiv.	# Samples
Saddleback tamarin, *Leontocebus weddelli*	8	♂	22	85
		♀	11	42
Emperor tamarin, *Saguinus imperator*	5	♂	10	39
		♀	14	49
Total individuals in analysis			**57**	Total animal samples 215
				Air and blank samples 63
				Total samples 278

Peaks with similar retention times in different chromatograms could represent the same, or different, compounds. I decided which of the two possibilities applied by comparing the mass spectra of individual chromatographic peaks, as illustrated in **Fig. IV.3**. Visual inspection of the

chromatograms of the 215 animal samples collected (from the anogenital, suprapubic, and sternal scent-glands, and the inner thigh skin) permitted the recognition of a number of peaks that were absent in all the controls (i.e. the air samples, the blank samples, or the fibre blanks). The majority of these peaks, however, were poorly resolved, i.e. they represented a superposition of two or more compounds of similar m/z, visible through a very complex mass spectrum. It was thus only possible to provide a tentative identification of 11 compounds (i.e. compounds A–K). These compounds, referred to here as ‘compounds of interest’, were present in 33 of the samples (**Table D.1**).

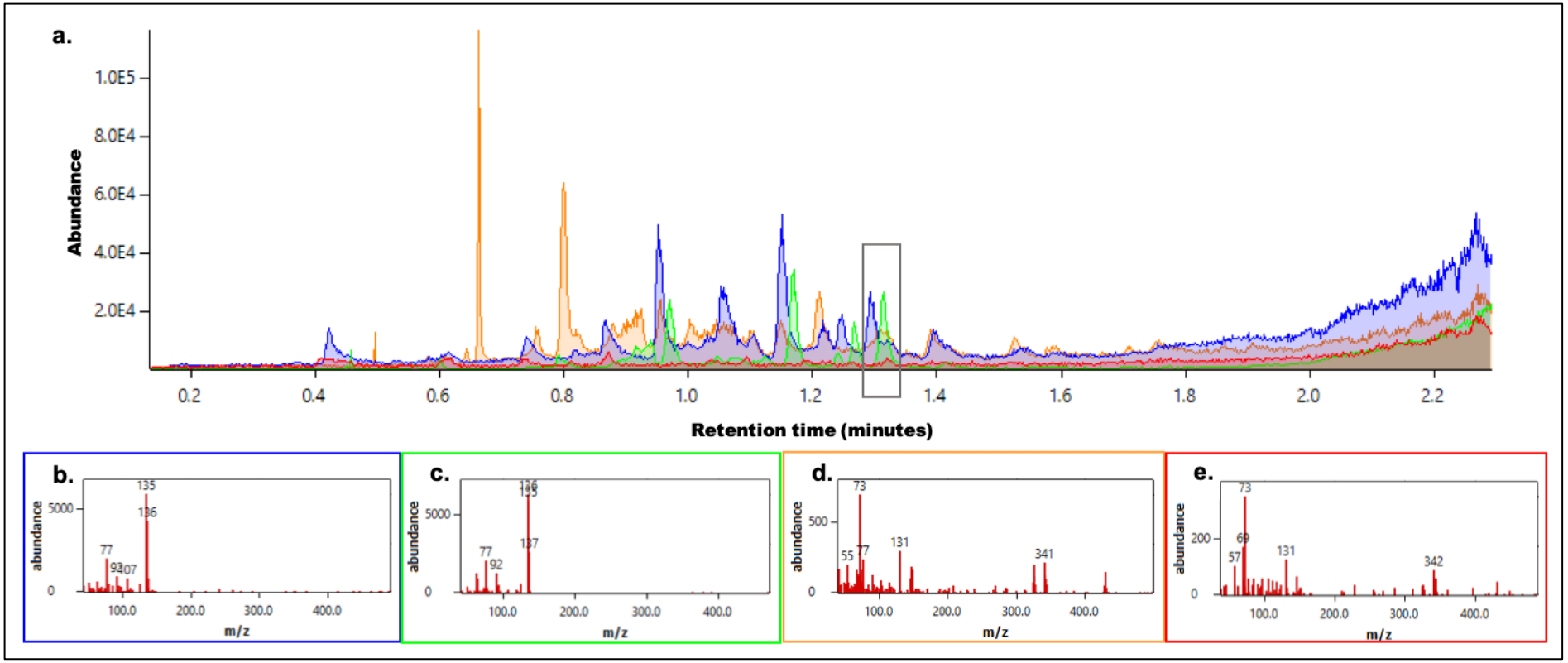


Figure IV.3. a. Overlaid chromatograms of the anogenital (blue), and suprapubic (green) gland swabs of two female emperor tamarins, the sternal gland swab of a male saddleback tamarin (orange), and a fibre blank ran at the same time as the samples (red), analysed using the Torion® portable GC-MS. The mass spectra **b.**, **c.**, **d.**, and **e.** correspond to the peaks of respective colours framed in grey, the retention times of which were similar enough to qualify for being the same compound. Peaks **b.** and **c.**, of nearly identical mass spectra, were selected as a compound of interest; whereas peak **d.**, which was very similar to the blank peak **e.**, was discarded as a likely contaminant.

Compounds A, B, D, G, H, I and J were found in at least two different samples, while compounds C, E, F and K appeared to be unique to an individual (see **Appendix D**, **Table D.1**). Only compound D was found in both species; compounds A, B, C, E, F, H and K were specific to saddleback tamarins, the other three to emperor tamarins (**Fig. IV.4a**; **Table D.1**). The majority of compounds were only retrieved from one or two groups; however compound A was found in six out of the eight saddleback tamarin groups, and compound G from three out of the five emperor tamarin groups (**Table D.1**). Compounds A, B, D, G, and H were found in both sexes, while compounds C, E, and F were male-specific, and compounds I, J and K were female-specific (**Fig. IV.4b**; **Table D.1**). Most of the compounds were exclusively found in primary breeders; compounds A, G, and J were also present in samples from secondary breeders; and compound F was only found in a secondary breeder (**Fig. IV.4c**; **Table D.1**). Compound A was unique in being found principally in secondary breeding males. Samples from individual tamarins generally contained only one compound of interest, and compounds C, E, F and K were only found in single animals (**Table D.1**). Nevertheless, compounds A and F, and compounds B and C, were retrieved from the samples of a secondary breeding male saddleback tamarin (LW2_M1), and a primary breeding male saddleback tamarin (LW6_M4), respectively; and compounds D, I, and J were found in samples from a primary breeding female emperor tamarin (SI2_F1; **Table D.1**). Finally, compounds C, D, E and G were only found on anogenital gland swabs, while compounds J and K were only on suprapubic gland swabs, and compound F on a sternal gland swab (**Fig. IV.4d**; **Table D.1**). Compounds A, B, and H were retrieved from all areas sampled, including the skin, suggesting a non-specialized glandular compound, or even an exogenous origin.

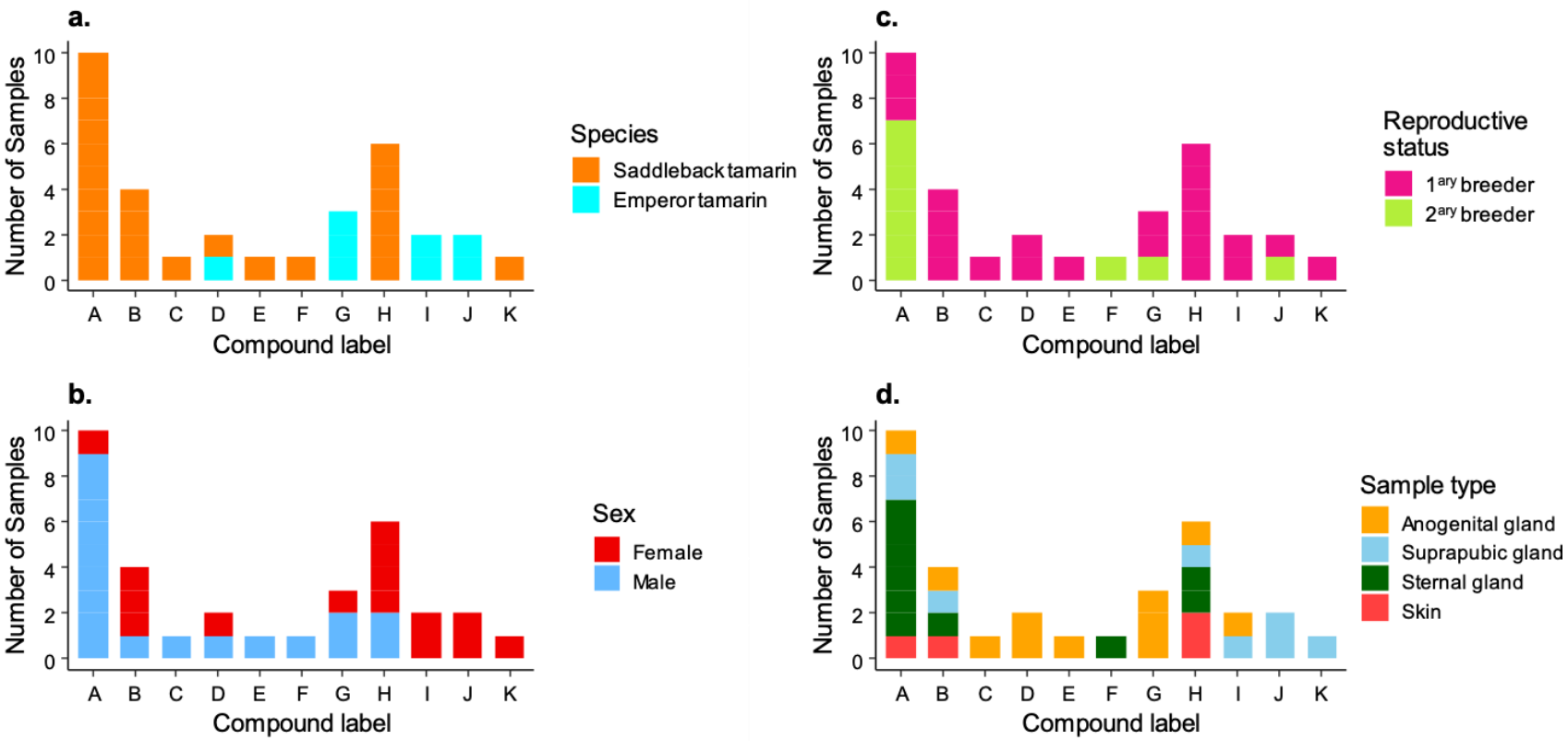


Figure IV.4. Distribution of the 11 compounds of interest obtained *in situ*, between the different categories of **a.** species, **b.** sex, **c.** reproductive status (i.e. primary and secondary breeders only, as nonbreeder samples did not show any of the compounds of interest), and **d.** sample type.

Anogenital, suprapubic, sternal scent-glands, and the inner thigh skin region, respectively accounted for 34%, 24%, 30%, and 12% of the pool of samples containing one or several of the 11 compounds of interest (**Fig. IV.5**). Anogenital and suprapubic scent-gland samples from both species showed compounds of interest, while sternal scent gland and skin samples from saddleback tamarins only, presented compounds of interest (**Fig. IV.5a**). Scent-gland samples containing compounds of interest were represented in both sexes, although the majority of compounds of interest in suprapubic, and sternal gland samples, were from females, and males, respectively (**Fig. IV.5a**). Similarly, compounds of interest from all three scent-gland types were retrieved from both primary and secondary breeders, although the majority of compounds of interest from anogenital gland samples were from primary breeders (**Fig. IV.5b**). Compounds of interest retrieved from skin samples were only found in primary breeders.

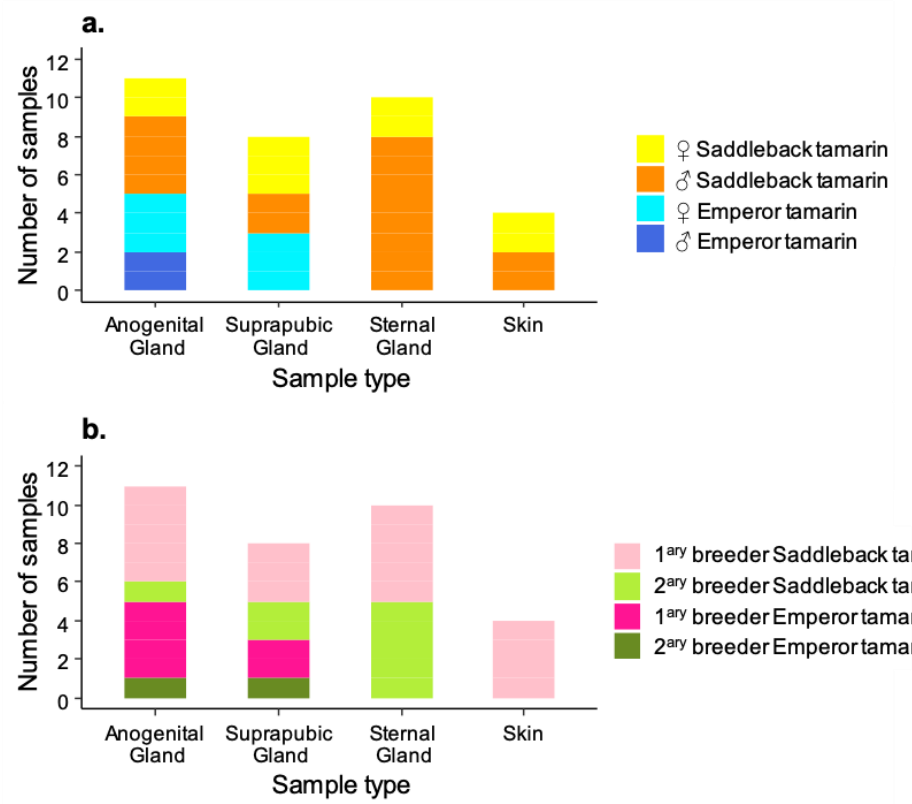


Figure IV.5. Distribution of the four types of sample presenting a compound of interest, between **a.** species and sexes, and **b.** species and reproductive status (i.e. primary and secondary breeders only, as nonbreeder samples did not show any of the compounds of interest).

Additionally, the likelihood of presence of compounds of interest in the samples was tested across categories of species, sex, reproductive status, and sample type. It was significant at the levels of species (Kruskal-Wallis rank sum test: χ^2=5.286, *df*=1, *P*= 0.022) and reproductive status (χ^2=10.360, *df*=2, *P*= 0.006; **Table IV.2**). In particular, saddleback tamarin samples were more likely to contain a compound of interest than emperor tamarin samples (Dunn's post-hoc test: *Z*=2.180, *P*= 0.029), and samples from primary breeders were more likely to show compounds of interest than samples from nonbreeders (*Z*=3.167, *P*= 0.005; **Table IV.2**).

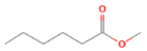
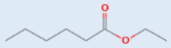
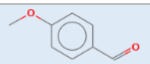
Table IV.2. Results of Kruskal-Wallis sum rank tests of difference, and Dunn's post-hoc tests on pairwise comparisons, on the likelihood of presence of compounds of interest in the samples analysed *in situ*, across categories of species, sex, reproductive status, and sample type. χ^2= χ^2-statistic; *df*= degrees of freedom; *Z*= *Z*-statistic; and *P*= p-value, significant at *P*≤ 0.05 (in bold).

Category tested	Kruskal-Wallis rank sum test	Pairwise comparisons	Dunn's post-hoc test
Species	χ^2= 4.739, *df*= 1, ***P*= 0.029**	Emperor tamarin – Saddleback tamarin	*Z*= 2.180, ***P*= 0.029**
Sex	χ^2= 0.107, *df*= 1, *P*= 0.744	Male – Female	*Z*= 0.327, *P*= 0.740
Reproductive status	χ^2= 10.099, *df*= 2, ***P*= 0.006**	1_{ary} – 2_{ary}	*Z*= 1.269, *P*= 0.613
		1_{ary} – None	*Z*= 3.167, ***P*= 0.005**
		2_{ary} – None	*Z*= 1.986, *P*= 0.141
Sample type	χ^2= 4.408, *df*= 3, *P*= 0.223	Anogenital – Suprapubic	*Z*= 0.896, *P*= 1.000
		Anogenital – Sternal	*Z*= 0.321, *P*= 1.000
		Anogenital – Skin	*Z*= 1.946, *P*= 0.310
		Suprapubic – Sternal	*Z*= 0.576, *P*= 1.000
		Suprapubic – Skin	*Z*= 1.060, *P*= 1.000
		Sternal – Skin	*Z*= 1.631, *P*= 0.620

IV.3.1.2. Identity of compounds of interest in wild tamarin scent-gland and skin samples analysed *in situ*

Automatic search in the NIST mass spectral library, together with visual inspection of the mass spectra and retention times of the peaks of interest, permitted the tentative identification of some of the compounds characterized by these peaks: methyl hexanoate (B), benzaldehyde (D), ethyl hexanoate (E), acetophenone (F), a branched C15 alkane (G), 4-methoxybenzaldehyde (I), and hexadecan-1-ol (K; **Table IV.3**). However, unlike the captive callitrichid samples analysed in the laboratory (see **Chapter 3**, section III.3.3), these identifications could not be validated, as I was unable to compare the retention times with those of commercially obtained compounds on the Torion® GC-MS. Moreover, I was unsuccessful at assigning an identity to compounds A, C, H and J, due to high peak impurity (i.e. the peaks detected by the GC-MS were not fully resolved, hence their mass spectra were likely composed of more than one compound, impossible to tell apart).

Table IV.3. Tentative identity of the 11 compounds of interest retrieved from wild tamarin scent-gland samples analysed *in situ*. Identity was achieved by automated search on the NIST mass spectral library, followed by visual inspection of the peaks' mass spectra. RT= retention time in minutes; SD= standard deviation. Diagrams of the compounds' chemical structure are reproduced from the NIST chemistry WebBook (www.webbook.nist.gov).

Compound label	Mean RT ±SD	Candidate compound identity	Functional group	Chemical structure	Prevalence in samples†	Also found in captive study?
A	0.757 ±0.008	Unidentified, probably of non-mammalian origin	Unk.	Unk.	10	NA
B	0.804 ±0.001	Methyl hexanoate	Ester		4	✗
C	0.849 ±0.000	Unidentified	Unk.	Unk.	1	NA
D	0.868 ±0.003	Benzaldehyde	Aromatic aldehyde		2	✓
E	0.900 ±0.000	Ethyl hexanoate	Ester		1	✗
F	1.043 ±0.000	Acetophenone	Aromatic ketone		1	✓
G	1.066 ±0.014	Branched C15 alkane	Alkane	Unk.	3	✗
H	1.300 ±0.004	Unidentified carboxylic acid	Carboxylic acid	Unk.	6	NA
I	1.304 ±0.015	4-Methoxybenzaldehyde	Aromatic aldehyde		2	✓
J	1.321 ±0.002	Unidentified	Unk.	Unk.	2	✗
K	1.551 ±0.000	Hexadecan-1-ol	Alcohol		1	✗

† Prevalence is the number of samples containing the compound of interest.

IV.3.2. Laboratory analyses of wild emperor tamarin samples

IV.3.2.1. Chemical differences at the levels of group, sex, reproductive status and the individual, and between scent-glands

A total of 27 swabs of anogenital, suprapubic, and sternal scent-glands, as well as swabs of the skin of the inner arm (skin), were additionally collected from eight wild emperor tamarins at EBLA during FPI's 2018 field season (see **Appendix E**, **Table E.1**), and transported for analyses in the laboratory at ARU. Two air samples collected at the same time as the animal samples, and several blanks (i.e. empty vial, empty fibre), were also run together with the animal samples. Visual inspection of the chromatograms permitted tentative identification of 25 compounds of interest present in 25 of the samples, but not in any of the blanks (**Table E.1**).

The majority of the selected compounds of interest were unique to a sample (56%), and only three were found in at least eight samples. In addition, most of the compounds of interest were present solely in group SI7 (56%), only represented by 11 samples from three animals, while five compounds were specific to group SI6 (20%; **Fig. IV.6a**). Five of the compounds were retrieved only from females (20%), and 12 only from males (48%; **Fig. IV.6b**). The majority of compounds were unique to secondary breeders (88%), and no compound was specific to the only primary breeder (**Fig. IV.6c**). Several compounds were retrieved from the samples of each individual emperor tamarin, ranging from 2–13 compounds of interest per individual (**Table E.1**). Finally, nine compounds were found exclusively in anogenital gland swabs (36%), one in suprapubic gland swabs (4%), six in sternal gland swabs (24%), but none in skin swabs only (**Fig. IV.6d**). Only two compounds were retrieved from all four sample types (8%). The variation in the occurrence of compounds of interest across categories of group, sex, reproductive status, and sample type, was non-significant (Pearson's χ^2 tests of difference; **Table IV.4**).

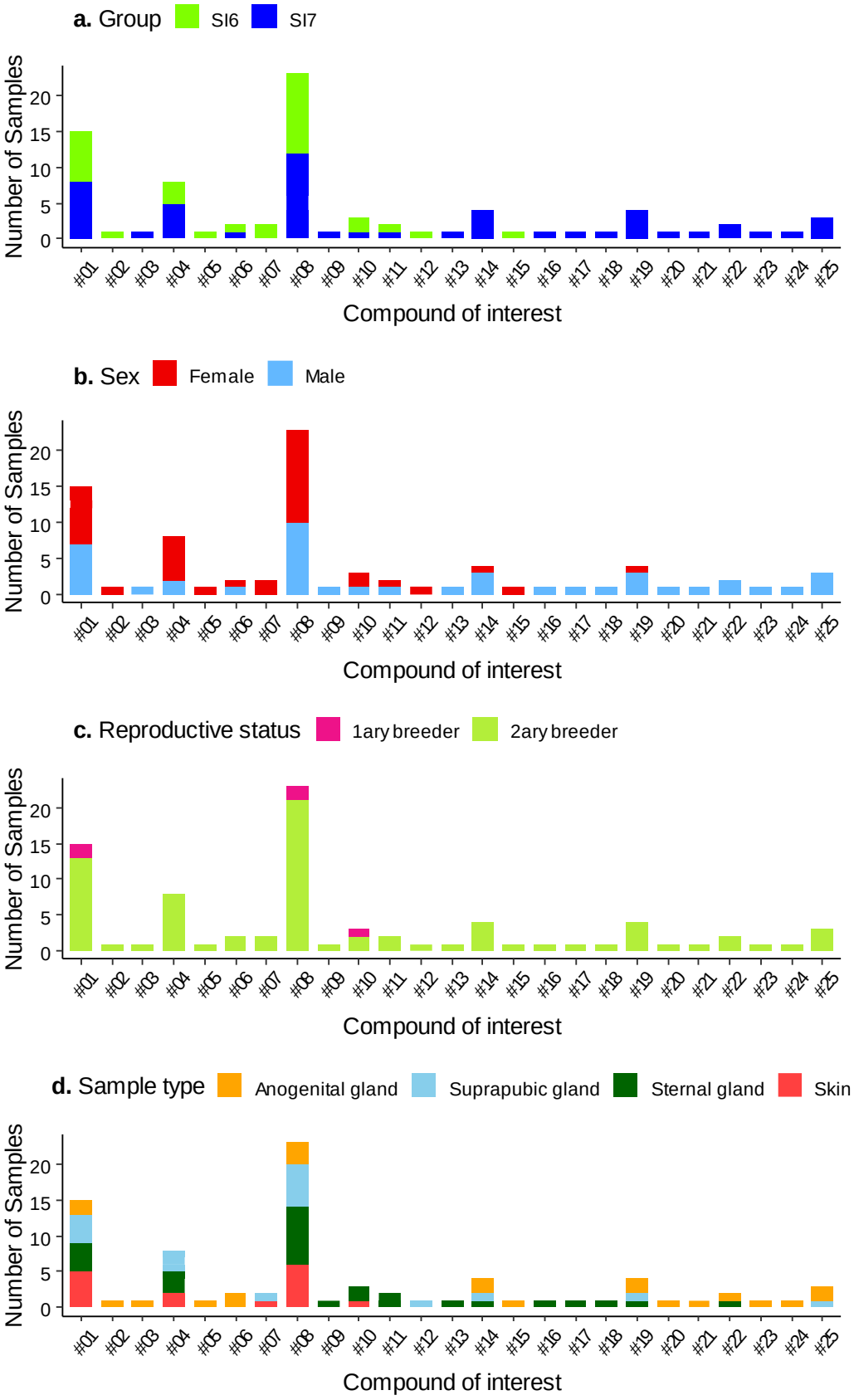


Figure IV.6. Distribution of the 25 compounds of interest retrieved by laboratory analyses, between the different categories of **a.** group, **b.** sex, **c.** reproductive status, and **d.** sample type.

Table IV.4. Results of Pearson's χ^2 tests with Yates' continuity correction, on the differences of distribution of the 25 compounds of interest across emperor tamarin groups, sexes, reproductive status, and sample types. χ^2= χ^2-Statistic, *df*= degrees of freedom, *P*= *P*-value, significant at $P< 0.05$.

Category tested	Pearson's χ^2 test
Group	χ^2= 26.651, *df*= 24 , *P*= 0.321
Sex	χ^2= 25.657, *df*= 24 , *P*= 0.371
Reproductive status	χ^2= 8.192, *df*= 24 , *P*= 0.999
Sample type	χ^2= 68.167, *df*= 72 , *P*= 0.606

IV.3.2.2. Identity of compounds of interest in wild tamarin scent-gland and skin samples analysed in the laboratory

Automatic search in the NIST mass spectral library, together with visual inspection of the mass spectra and retention times of the peaks of interest, allowed most of the 25 compounds of interest to be tentatively identified (**Table IV.5**). As the analytical method employed here was the same as in the captive study, it was also possible to verify the identity of some of the compounds common to those found in the captive samples, by direct comparison of their retention times with those of commercially obtained compounds (see **Chapter 3**, section III.3.3.1). Hence, identity was verified for butan-1-ol (#01), benzaldehyde (#06), 1-octen-3-ol (#07), acetophenone (#10), and 4-methoxybenzaldehyde (#15). Identity of the rest of the compounds of interest remained tentative. The three most prevalent compounds were butan-1-ol (#01), 2-butoxyethanol (#04), and a branched C12 alkane (#08), retrieved from 15, eight, and 23 samples, respectively (**Table IV.5**).

Table IV.5. Tentative identity of the 25 compounds of interest retrieved from wild emperor tamarin scent-gland samples analysed in the laboratory. Identity was achieved by automated search on the NIST mass spectral library, followed by visual inspection of the peaks' mass spectra. Identity of the five compounds marked with an asterisk was confirmed by comparison of their retention times with those of commercially obtained compounds. RT= retention time in minutes, SD= standard deviation. Diagrams of compounds' chemical structure are reproduced from the NIST chemistry WebBook (www.webbook.nist.gov).

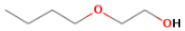
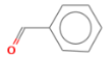
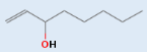
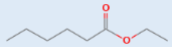
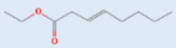
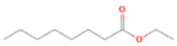
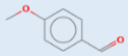
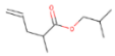
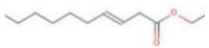
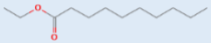
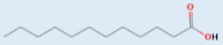
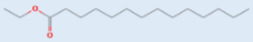
#	Mean RT (±SD)	Candidate compound identity	Functional group	Chemical structure	Prevalence in samples†	Also found in captive study?	Also found in *in situ* analyses?
01	3.016 ±0.010	Butan-1-ol⋆	Alcohol		15	✓	×
02	4.044 ±0.000	3-Hydroxybutan-2-one	α-hydroxyketone		1	×	×
03	4.411 ±0.000	3-Methylbutan-1-ol	Alcohol		1	×	×
04	9.535 ±0.010	2-Butoxyethanol	Alcohol		8	×	×
05	10.793 ±0.000	Branched C9 alcohol	Alcohol	Unk.	1	×	×
06	11.125 ±0.007	Benzaldehyde⋆	Aromatic aldehyde		2	✓	✓
07	11.759 ±0.002	1-Octen-3-ol⋆	Unsaturated alcohol		2	✓	×
08	11.940 ±0.006	Branched C12 alkane	Alkane	Unk.	23	×	×
09	12.315 ±0.000	Ethyl hexanoate	Ester		1	×	✓
10	14.221 ±0.003	Acetophenone⋆	Aromatic ketone		3	✓	✓
11	16.396 ±0.010	Branched C7 carboxylic acid	Carboxylic acid	Unk.	2	×	×
12	17.239 ±0.000	Branched C8 carboxylic acid	Carboxylic acid	Unk.	1	✓	×

Table IV.5. Continued.

#	Mean RT (±SD)	Candidate compound identity	Functional group	Chemical structure	Prevalence in samples†	Also found in captive study?	Also found in *in situ* analyses?
13	17.401 ±0.000	Ethyl oct-3-enoate	Unsaturated ester		1	×	×
14	17.589 ±0.004	Ethyl octanoate	Ester		4	×	×
15	19.096 ±0.000	4-Methoxybenzaldehyde⋆	Multifunctional compound		1	✓	✓
16	21.606 ±0.000	Butyl 2-methylpent-4-enoate	Unsaturated ester		1	×	×
17	21.694 ±0.000	Decanoic acid	Carboxylic acid		1	×	×
18	21.782 ±0.000	Ethyl dec-3-enoate	Unsaturated ester		1	×	×
19	22.184 ±0.003	Ethyl decanoate	Ester		4	×	×
20	23.893 ±0.000	Cyclododecane	Cycloalkane		1	✓	×
21	25.743 ±0.000	Dodecanoic acid	Carboxylic acid		1	×	×
22	26.281 ±0.002	Ethyl dodecanoate	Ester		2	×	×
23	29.972 ±0.000	Ethyl tetradecanoate	Ester		1	×	×
24	31.221 ±0.000	Ethyl pentadecanoate	Ester		1	×	×
25	33.980 ±0.003	Unknown ethyl ester	Ester	Unk.	3	×	×

† Prevalence is the number of samples containing the compound of interest.

IV.4. Discussion

IV.4.1. Chemical signatures in wild tamarin scent-gland secretions

The present study revealed differences in the chemical composition of material swabbed from the scent-glands and skin of wild emperor and saddleback tamarins at the levels of species, groups, sex, reproductive status, the individual, and between sample types (i.e. anogenital, suprapubic, and sternal glands, and the skin from the inner thigh or arm). Using the Torion® GC-MS for *in situ* chemical analyses of 218 wild tamarin samples, I was able to tentatively identify 11 putative tamarin semiochemical compounds. Furthermore, I retrieved 25 compounds of interest from 27 additional wild emperor tamarin scent-gland samples, transported back to ARU and analysed in the laboratory.

The compounds retrieved from the wild tamarin scent-gland samples analysed *in situ* appeared to be species-specific, as only one (i.e. benzaldehyde [D]), out of the 11 compounds of interest, was found in both tamarin species. This suggests a conspicuous species chemical signature, and even potential involvement of different biosynthetic pathways, in emperor and saddleback tamarins. Nevertheless, at EBLA these two sympatric tamarin species regularly form mixed-species foraging troops. There is evidence that the two species recognize each other's calls, despite their being quite different, and that individuals of one species use vocal cues from the other species in foraging and predator avoidance (Windfelder, 2001). This has also been established in other sympatric tamarin associations such as between Geoffroy's saddleback tamarins, *Leontocebus nigrifrons* (formerly *Saguinus fuscicollis*, recently reassigned, Rylands et al., 2016), and moustached tamarins, *Saguinus mystax* (Smith, 1997). Besides, emperor and saddleback tamarins at EBLA are often observed sniffing and overmarking in response to scent-marks from the other species (Watsa, 2013; pers. obs.). These sympatric tamarins may very well recognize and respond to each other's scent signals despite the differences in their chemical composition.

All groups of both species of tamarin presented at least one sample containing a compound of interest. Most compounds were common to samples from several groups, suggesting a similarity between groups

sharing the same compound. For instance, samples from SI2 and SI4 shared ethyl hexanoate (E) and a branched C15 alkane (G), and samples from SI1 and SI5 shared the unidentified compound H, suggesting that these groups are more closely related to each other, than they are with the other emperor tamarin groups with which they do not share compounds. Indeed, home ranges of emperor tamarin groups SI2 and SI4, and SI1 and SI5, respectively, are closer to each other geographically than they are to any of the other groups. Neighbouring groups are more likely to exchange individuals; for example it is not uncommon for a young female to disperse from her native group and associate with males from neighbouring groups (Watsa, 2013). This could result in a more similar chemical signature between these groups, owing to the fact that they share similar commensal bacteria communities (Theis et al., 2013) and genetic pool, especially at the major histocompatibility complex (Charpentier, Boulet and Drea, 2008; Knapp, Robson and Waterhouse, 2006; Setchell et al., 2011). Unidentified compound A was found in samples from all but two of the saddleback tamarin groups, while methyl hexanoate (B) and unidentified compound C were only found in samples from group LW6, and unidentified compound H in group LW8. While compound A appears species-specific, compounds B and C might belong to a given chemical signature of group LW6, and compound H of group LW8. Similarly, in the emperor tamarin samples analysed in the laboratory, five compounds were specific to group SI6, and 14 to SI7, while only eight compounds were common to both groups. The home ranges of these two groups were over 2 km apart (i.e. nearly the opposite sides of the area monitored by FPI at EBLA), which could explain why they did not share more compounds. Other studies have also found chemical signatures at the group level (e.g. in mandrills, Vaglio et al., 2016; Eurasian otters, *Lutra lutra*, Kean et al., 2017; and Bechstein's bats, *Myotis bechsteinii*, Safi and Kerth, 2003).

Three of the compounds of interest retrieved from the *in situ* analyses, i.e. 4-methoxybenzaldehyde (I), hexadecan-1-ol (K), and unidentified compound J, and four from the laboratory analyses, i.e. 3-hydroxybutan-2-one (#02), a branched C9 alcohol (#05), 1-octen-3-ol (#07), and a branched C8 carboxylic acid (#12), were only found in female samples; while three compounds from *in situ* analyses, i.e. ethyl hexanoate (E), acetophenone (F), and unidentified compound C, and 12 from laboratory analyses, i.e. 3-methylbutan-1-ol (#03), ethyl hexanoate (#09),

ethyl oct-3-enoate (#13), butyl 2-methylpent-4-enoate (#16), decanoic acid (#17), ethyl dec-3-enoate (#18), cyclododecane (#20), dodecanoic acid (#21), ethyl dodecanoate (#22), ethyl tetradecanoate (#23), ethyl pentadecanoate (#24) and an unknown ethyl ester (#25), were specific to male samples. Hence these putative tamarin compounds could be sex-specific, potentially involved in mate choice or intrasexual competition. However, the presence of 1-octen-3-ol, acetophenone, 4-methoxybenzaldehyde, and cyclododecane in multiple samples from both males and female captive tamarins (see **Chapter 3**, section III.3.3) suggests that these results may be an artefact of the small sample size in the present field study. A greater sample size will be needed to be able to confirm the specificity and function of given semiochemicals.

In tamarins, which are cooperative breeders, male reproductive status is difficult to determine, as all males of a group usually mate with the reproductive female(s) and help raise the offspring, regardless of whether they have achieved paternity (e.g. in moustached tamarins, Huck et al., 2005). As expected in a polyandrous mating system, several males per group were qualified as primary breeders in EBLA's study population. Female reproductive status, on the other hand, is easier to monitor, as pregnancy and nursing are visible externally. The dominant female of a group is often the only one to reproduce (Garber et al., 2016; Huck, Löttker and Heymann, 2004). Subordinate females, usually offspring of the dominant female, often show an absence of ovulation, and play the role of helpers in the family group. This originates from a suppressive effect of the dominant female, at behavioural and olfactory levels, and benefits the family unit by ensuring a higher survival rate of the offspring (e.g. in common marmosets, Abbott et al., 1998; Barrett, Abbott and George, 1990; Saltzman et al., 1997; Ziegler and Sousa, 2002; Ziegler, 2013; cotton-top tamarins, *S. oedipus*, Heistermann et al., 1989; Savage, Ziegler and Snowdon, 1988; and pygmy marmosets, *Cebuella pygmaea*, Spurlock, 2001; reviewed in Beehner and Lu, 2013). However in the study population, groups frequently possessed more than one primary breeding female, indicating a less straightforward relationship between reproduction and dominance (Watsa, Erkenswick and Robakis, 2017). Polygyny was reported in another wild population of saddleback tamarins (Calegaro-Marques, Bicca-Marques and Azevedo, 1995), as well as in wild groups of moustached tamarins, cotton-top tamarins, common marmosets, buffy-

tufted marmosets, *C. aurita*, buffy-headed marmosets, *C. flaviceps*, golden lion tamarins, *Leontopithecus rosalia*, and Goeldi's monkeys (reviewed in Smith et al., 2001a). Indeed, different reproductive strategies may be used by subordinate female tamarins, hence explaining variation in reproductive status, as was suggested in common marmosets (Arruda et al., 2005; Lazaro-Perea, 2001; Sousa et al., 2005; see **Chapter 2**, section II.4.1.1). In both species studied *in situ*, all but one of the female samples, and half of the male samples, presenting a compound of interest, originated from primary breeders. Therefore, methyl hexanoate (B), unidentified compound C, benzaldehyde (D), ethyl hexanoate (E), unidentified compound H, 4-methoxybenzaldehyde (I), and hexadecan-1-ol (K), which were exclusively retrieved from primary breeders, are likely to be associated with reproduction, mate choice and/or intrasexual competition (see **Chapter 2**, section II.4.1.1). In contrast, compounds retrieved from the samples analysed in the laboratory were for the most part uniquely found in secondary breeding emperor tamarins; and no compound was specific to primary breeders. However, only one of the eight individuals for which swabs were collected was a primary breeding male, which probably explains the over-representation of secondary breeders in the compounds list.

In samples analysed *in situ*, compound C, ethyl hexanoate (E), acetophenone (F), and hexadecane-1-ol (K) were found in single individual tamarins, which may indicate a role for these compounds in signalling individual identity. Moreover, methyl hexanoate (B) was retrieved from two different individuals belonging to group LW6, and compound H from two different individuals in group LW8. As seen earlier, animals belonging to the same family group have more closely related scents. These two compounds may hence constitute part of the chemical signature at the group level. Conversely, compound A, benzaldehyde (D), a branched C15 alkane (G), 4-methoxybenzaldehyde (I), and compound J, were common to two or more individuals from different groups, suggesting that they do not contribute in individual chemical signatures. In samples analysed in the laboratory, 3-hydroxybutan-2-one (#02), 3-methylbutan-1-ol (#03), a branched C9 alcohol (#05), ethyl hexanoate (#09), a branched C8 carboxylic acid (#12), ethyl oct-3-enoate (#13), 4-methoxybenzaldehyde (#15), butyl 2-methylpent-4-enoate (#16), decanoic acid (#17), ethyl dec-3-enoate (#18), cyclododecane (#20), dodecanoic acid (#21), ethyl

tetradecanoate (#23), and ethyl pentadecanoate (#24), were found in single individual emperor tamarins, hence these compounds may play a role in signalling individual identity in this species.

Finally, some of the compounds of interest were specific to one type of scent-gland, while others were retrieved from different scent-glands. This suggests that the chemical composition of tamarin glandular secretions has common characteristics, but also differences, between the anogenital, suprapubic, and sternal scent-gland areas. Histological analyses of the anogenital and suprapubic scent-gland skin of cotton-top tamarins (Fontani et al., 2014), the anogenital scent-gland of saddleback tamarins (Zeller et al., 1988), and the suprapubic and sternal scent-gland skin of lion tamarins, *Leontopithecus* spp. (Moraes et al., 2006), showed no difference between the tissues of these different glands. The same can therefore be expected for the two tamarin species studied here. Similar to the present results, Scordato, Dubay and Drea (2007) found chemical differences between swabs of labial, scrotal, and brachial scent-glands in ring-tailed lemurs; and Spence-Aizenberg et al. (2018) between swabs of pectoral and subcaudal glands in owl monkeys. In samples analysed *in situ*, unidentified compound A, methyl hexanoate (B), and unidentified compound H were retrieved from swabs of the inner thigh skin region of saddleback tamarins, as well as from scent-gland samples from different individuals. In samples analysed in the laboratory, butan-1-ol (#01), 2-butoxyethanol (#04), 1-octen-3-ol (#07), a branched C12 alkane (#08), and acetophenone (#10) were found in swabs of the inner arm of emperor tamarins as well as from glandular swabs. It may be argued that these compounds may then not originate from the scent-gland itself and should therefore be classified as a contaminant. Nevertheless, tamarins spend a lot of time in physical contact with each other, while sleeping in tree knots, and grooming (Smith et al., 2007). They also perform allomarking, where they deposit a scent-mark on the body of a conspecific (Lledo-Ferrer, Peláez and Heymann, 2010). It is therefore conceivable to find a glandular compound on another body part of the animal. As all compounds found on an animal's skin were also retrieved from a scent-gland in other individuals of the same species, I decided not to rule out these compounds from my list of compounds of interest. However, these results demonstrate that neither the inner thigh, nor the inner arm skin area, constitute good control regions to use for tamarin semiochemical studies.

IV.4.2. Identity of the compounds of interest retrieved from wild tamarin scent-gland samples and comparison with other studies

Identities of compounds of interest from the samples analysed *in situ* using the Torion® GC-MS, as well as those from the samples analysed in the laboratory for which verification by comparison with a commercially obtained compound was not possible, remain tentative. Benzaldehyde (D & #06), acetophenone (F & #10), and 4-methoxybenzaldehyde (I & #15) were found to be common compounds, retrieved from several samples analysed both *in situ* and in the laboratory, as well as from samples from suprapubic scent-glands or deposited scent-marks of captive emperor tamarins, cotton-top tamarins, and silvery marmosets, *Mico argentatus* (see **Chapter 3**, section III.3.3). In addition, ethyl hexanoate (E & #09) was retrieved from both a swab of anogenital gland of a male saddleback tamarin analysed *in situ*, and a swab of sternal gland of a male emperor tamarin analysed in the laboratory. Moreover, butan-1-ol (#01), 1-octen-3-ol (#07), a branched C8 carboxylic acid (#12) and cyclododecane (#20), which were retrieved from laboratory analyses of wild samples, were also found in samples from captive emperor tamarins, cotton-top tamarins, and silvery marmosets (see **Chapter 3**, section III.3.3).

The number of compounds retrieved from the 27 wild emperor tamarin scent-gland samples analysed in the laboratory (N=25 in total, ranging from 1–12 per sample) was fewer than found in the five captive emperor tamarin suprapubic scent-gland samples (N=37 in total, ranging from 6–20 per sample, of which 17 were selected as compounds of interest; see **Chapter 3**, section III.3.3.2.1), despite the fact that the exact same analytical method (i.e. SPME–GC-MS using the same instrument at ARU) was employed. Moreover, only seven compounds were common to the two datasets, i.e. butan-1-ol, benzaldehyde, 1-octen-3-ol, acetophenone, a branched C8 carboxylic acid, 4-methoxybenzaldehyde, and cyclododecane. Such diverging results suggest that captivity likely affects the chemical composition of emperor tamarin scent-gland secretions. Differences in diet, elements of the enclosure, and husbandry procedures, can influence the commensal bacteria communities in these captive primates, thus resulting in different compounds being produced (Archie and Theis, 2011; Clayton et al., 2016). Wild tamarins, which have access to a greater variety of food items, and potentially interact with a

greater diversity of organisms (i.e. con- and hetero-specifics, predators, prey, parasites and other microorganisms), could therefore be expected to produce more complex chemical signals. On the other hand, in an indoor zoo enclosure the air is often saturated with smells of the animals' excrement and urine, as well as food, mixed with those of neighbouring enclosures (pers. obs.), which could participate in the chemical blend sampled on the swab. Nonetheless, the variation in chemical composition between samples from wild and captive emperor tamarins may also originate from natural differences between the two populations. Indeed, even though both captive and wild emperor tamarins sampled belonged to the same subspecies, *S. i. subgrisescens*, important genetic differences can be expected between the two populations, potentially leading to chemical differences in their produced scents (see section IV.4.1). The fact that some compounds of interest were retrieved from both wild and captive samples indicates that captive conditions, including diet and environment, may not completely transform an animal's odour, which further legitimises the use of captive studies to help understand wild animals' chemosignalling.

Some of the compounds retrieved from the wild samples analysed in the laboratory, but not in the wild samples analysed *in situ*, nor in the captive samples, may have originated from the degradation of previous compounds during transportation of the samples. Indeed, owing to unforeseen logistical difficulties sample shipment between Peru and the UK was executed in several steps, during some of which the samples were left to thaw. As was experimentally tested in **Chapter 3**, sections III.3.4 and III.4.3, the chemical composition of this type of samples is likely to change over time when they are not kept frozen. For instance, the esters retrieved from the samples, ethyl decanoate (#19) and ethyl dodecanoate (#22), are frequent products from the esterification of their carboxylic acid precursors, decanoic acid (#17) and dodecanoic acid (#21), which were also found in the samples. Therefore, it is probable that these esters, as well as ethyl hexanoate (#09), ethyl octanoate (#14), ethyl tetradecanoate (#23), and ethyl pentadecanoate (#24) also retrieved from the samples, originate from chemical reactions inside the sample vial after swab collection. In addition, many compounds were potentially lost during transportation of the samples at room temperature, which could explain why fewer compounds were retrieved from the wild samples than from the captive samples. Spence-

Aizenberg et al. (2018) reached the same conclusions, after finding that subcaudal and pectoral scent-gland samples of wild owl monkeys, *A. azarae*, contained fewer compounds than those of captive animals of the same genus. They had also transported their samples at room temperature. Importantly, as the number of scent-gland samples collected in captivity was very small, increasing sample size will be necessary to further understand the influence of captive and wild conditions on the chemical composition of primate scent-gland secretions.

All wild tamarin semiochemicals tentatively identified from *in situ* analyses, and most of those identified from laboratory analyses, have been reported before as candidate mammalian semiochemicals (see **Chapter 5**, **Table V.1** for an exhaustive review of the mammalian semiochemicals common to the compounds found in the present study). Nevertheless, some compounds are also known to be produced by plants and bacteria (https://pubchem.ncbi.nlm.nih.gov; www.pherobase.com), which suggests they might not have been directly produced by the animals. This was for instance the case for 3-hydroxybutan-2-one (#02) and 3-methylbutan-1-ol (#03), which were reported to be produced by plants, and were retrieved from cultures of human commensal bacteria (i.e. *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*, Tait et al., 2014). However, as seen in **Chapter 3**, section III.4.2, it is possible that a compound of plant origin, derived from alimentation, or produced by commensal bacteria present on the scent-gland, serves as a mammalian semiochemical (e.g. in greater sac-winged bats, *Saccopteryx bilineata*, Voigt, Caspers and Speck, 2005; meerkats, *Suricata suricatta*, Leclaire, Nielsen and Drea, 2014; Leclaire et al., 2017; and spotted hyenas, Theis, Schmidt and Holekamp, 2012; Theis et al., 2013).

IV.4.3. Limitations in the identification of putative wild tamarin semiochemicals, and future directions

As recommended by Charpentier et al. (2012) in their critical review of the chemical ecology of mammalian communication, great care must be given when attempting to identify a particular compound as a semiochemical. Many potential sources of contamination may occur at the time of scent-mark deposition and sample collection, storage, or extraction,

which can make it considerably difficult to establish whether a chemical detected ***i.*** really originates from the animal's secretion, ***ii.*** is actually present in the sample, not an artefact of contamination during the analysis, and ***iii.*** is truly the chemical targeted by the mass spectral library search. Therefore, in the chemical analyses presented here, the identity, and potential specificity across categories of samples (e.g. group- or sex-specific), of many compounds of very low prevalence in the samples must be considered with great caution. Repeated samples should help minimize many of these potential confounding aspects. However, even two swabs taken consecutively from the same gland area might contain different compounds, as the scent-gland is continuously producing secretions. Ideally, the presence of a certain compound in a natural secretion should be validated by replicating the chemical analysis using the authentic compound, as was done for some of the compounds of interest identified in the captive callitrichid semiochemistry study in **Chapter 3** (section III.3.3.1). Additional validation should be sought by performing a behavioural bioassay to assess the response of conspecifics to this particular compound (see **Chapter 1**, section I.2.1.2). A particular compound may be genuinely a component of glandular secretion, and yet show no signalling role, i.e. not be a semiochemical. Such bioassays have been carried out in captive primates, such as in common marmosets (Addessi, Chiarotti and Visalberghi, 2007; Smith and Abbott, 1998), ring-tailed lemurs (Scordato and Drea, 2007), rhesus macaques, *Macaca mulatta* (Henkel et al., 2015), and Japanese macaques, *M. fuscata* (Rigaill et al., 2017). In natural conditions however, conducting behavioural bioassays is more challenging. Thanks to the habituation work carried out by FPI at EBLA, future work on scent communication in this tamarin population will include behavioural bioassays.

The use of the Torion® GC-MS for the analyses of tamarin semiochemicals was principally motivated by its portability. This study was, to the best of my knowledge, the first attempt to analyse animal scent chemical composition using this instrument. Nevertheless, the number of compounds of interest retrieved from the *in situ* analyses (N=11 compounds) was quite limited when compared with the laboratory analyses (N=25 compounds), despite the fact that over eight times more samples were analysed in the *in situ* conditions (N=218 samples) than the laboratory conditions (N=27 samples). This suggests that the capacity of the

stationary phase of the Torion® gas chromatograph to separate individual compounds, and/or the sensitivity of the Torion® mass spectrometer, was lower than that of the bench-top instrument used in the laboratory at ARU. Similarly Kücklich et al. (2017), who compared the performance of a portable GC-MS device containing a thermal desorption trap, to that of cotton swabs analysed on a bench-top GC-MS, for the analysis of body odour of captive common marmosets, found that the portable GC-MS retrieved fewer compounds. Moreover, although the Custodion® SPME fibre used for sample extraction with the Torion® GC-MS was of identical composition as the Supelco SPME fibre used in the laboratory; the conditions of sample extraction time and temperature were different, which may have added to the variation observed between the two sets of analytical results. Nonetheless, a number of methodological aspects can readily be optimized to ensure greater success in future analyses of mammalian semiochemicals using the Torion® GC-MS. This includes improving sampling procedures, to limit the incorporation of volatile contaminants from the chemicals, and humans, present during sample collection and extraction. Incidentally, two of the most prevalent compounds retrieved from the swabs of wild tamarins analysed in the laboratory were subsequently identified as 2-phenoxyethanol and diethyltoluamide (DEET), two main components of insect repellent products, most likely originating from the clothes of the researchers conducting sample collection. Further optimization of the instrument working conditions (e.g. power generation, helium connection, availability of disposable material replacements), and preparation for on-site troubleshooting, will be necessary. A recommendation to future users of the Torion® GC-MS in isolated field conditions would be to carefully plan for all the technical challenges of their study. For example, the present project suffered from the fact that airline companies prohibited the transport of the Torion®-specific battery and helium canisters. This forced me instead to rely on a generic helium tank, which was costly and difficult to fit to the Torion® system, and a portable fuel generator, which released fumes that were likely responsible for a large hydrocarbon tail in the resulting chromatograms, thus preventing any interpretation of peaks of higher retention time. Future work using the Torion®, still a novel instrument, will take into account this preliminary experience.

IV.4.4. Conclusion

The present study revealed chemical signatures in scent-gland and skin swabs from wild sympatric tamarins at the levels of species, group, sex, reproductive status, and the individual, and between scent-gland types. Moreover, a number of compounds retrieved from the wild samples were tentatively identified as putative tamarin semiochemicals. Although preliminary, as a result of a relatively small sample size, a lack of repeatability, and the methodological problems encountered, this study provides the first attempt at analysing the chemical composition of wild tamarin scent-gland secretion samples *in situ* using the Torion® GC-MS. The use of this portable instrument was not as effective as the analyses of similar samples using a conventional bench-top GC-MS, after transporting the samples back to the UK. This laboratory analysis of wild samples, in turn, revealed a lower number of compounds identified as putative tamarin semiochemicals, than found in captive samples from the same species, analysed using the same methods. Furthermore, an important variation in sample chemical composition was found between the three types of analyses, which may originate from differences in sample storage and transportation. Nevertheless, portable GC-MS instruments have considerable potential to aid the study of primate olfactory communication in field conditions, and I hope the present results will provide help in this direction.

Chapter V – General discussion

V.1. Summary of the results

V.1.1. Correspondence between scent-marking behaviour and semiochemistry in captive callitrichids

V.1.1.1. Variation at the levels of species and group

In **Chapter 2**, I presented evidence for differences in the scent-marking behaviour of three species of captive callitrichids (see **Q1** in **Chapter 1**, section I.4.2). Cotton-top tamarins, *Saguinus oedipus*, scent-marked more frequently than emperor tamarins, *S. imperator*, and silvery marmosets, *Mico argentatus*. In addition, silvery marmosets often combined scent-marking with tree-gouging, a common foraging activity in this species, absent in tamarins (Rylands and Mittermeier, 2013). Moreover, silvery marmosets marked mainly using their suprapubic gland, in short depositions, while tamarins of both species principally deposited anogenital scent-marks, of variable duration. Differences in scent-marking behaviour have been documented in other callitrichid species both in the wild (e.g. in moustached tamarins, *S. mystax*, and Geoffroy's saddleback tamarins, *Leontocebus nigrifrons*, formerly *S. fuscicollis*, recently reassigned, Rylands et al., 2016) and in captivity (e.g. in common marmosets, *Callithrix jacchus*, Epple, 1970; and red-bellied tamarins, *S. labiatus*, Smith and Gordon, 2002). Such variation across species may reflect different strategies of olfactory communication, and maybe even differences in the relative importance of chemosignalling compared with other communication modalities (Higham and Hebets, 2013). In the semiochemical analyses conducted in **Chapter 3**, I further revealed a greater chemical richness in samples from silvery marmosets than from both tamarin species (see **Q6** & **Q9** in **Chapter 1**, section I.4.2). Samples from emperor and cotton-top tamarins did not differ in their chemical richness; nevertheless, their chemical diversity differed, suggesting a species-specific chemical signature, and thus potential involvement of different biosynthetic pathways in these different callitrichid species. Similar disparities have been found in other primate taxa (e.g. among owl monkeys, *Aotus* spp. Spence-Aizenberg et al., 2018; and brown lemurs, *Eulemur* spp., DelBarco-Trillo et al., 2011), as well as in other mammalian

taxa (e.g. among mustelids, *Mustela* spp., Zhang et al., 2002; and large felids, *Panthera* spp., Soini et al., 2012). Such interspecific variation in chemosignalling at behavioural and chemical levels supports the idea that different strategies of communication may be used by different species. This could help ensure that signals are conveyed to the intended receivers, which is especially relevant for species living sympatrically.

In addition, I revealed differences between groups of tamarins housed at different sites (see **Q1** & **Q6** in **Chapter 1**, section I.4.2): cotton-top tamarins at DMP scent-marked more often than the other groups of both emperor and cotton-top tamarins, although the chemical richness of their scent-marks was not significantly different. Samples from emperor tamarins at DMP had a lower chemical richness than those from the other callitrichid groups, in particular the emperor tamarins at TZ. Importantly, samples from all five groups displayed quite dissimilar chemical diversity. Other studies have also shown differences between groups at both behavioural level (e.g. in common marmosets, Epple, 1970; and yellow mongooses, *Cynictis penicillata*, Le Roux, Cherry and Manser, 2008), and chemical level (e.g. in mandrills, *Mandrillus sphinx*, Vaglio et al., 2016; Bechstein's bats, *Myotis bechsteinii*, Safi and Kerth, 2003; Eurasian otters, Kean et al., 2017; and spotted hyenas, *Crocuta crocuta*, Theis, Schmidt and Holekamp, 2012). These results suggest that group size and composition, as well as the characteristics of captive conditions (e.g. variations in diet, husbandry practices, temperature and illumination), likely play an important role in chemosignalling activity in captive animals.

V.1.1.2. Variation at the levels of sex, reproductive status, and the individual

My study indicated a variable effect of sex, reproductive status, and the individual, on callitrichid scent-marking behaviour (see **Q1** in **Chapter 1**, section I.4.2). Females marked more than males overall, although some males marked as much, or more, than females. Other studies have reported scent-marking activity to be performed mainly by female callitrichids (e.g. French and Snowdon, 1981; Miller, Laszlo and Dietz, 2003; Smith and Gordon, 2002), suggesting that it plays an important role in the advertisement of female reproductive state and mate choice (Heymann, 2006a). Yet other studies found no differences between sexes

(e.g. Lazaro-Perea, Snowdon and Arruda, 1999; Oliveira and Macedo, 2010). In many animal species, males use scent-marking more frequently than females, particularly to signal territory and dominance (Albone and Shirley, 1984). In callitrichid scent-marking in males has also been suggested to play a role in mate choice and to serve as a means of chemical mate guarding (Huck, Löttker and Heymann, 2004; Lledo-Ferrer, Peláez and Heymann, 2010).

As was the case for sex, reproductive status significantly influenced scent-marking overall, although this varied between groups. Either the reproductive male, the reproductive female, or the subordinate female of a group, was the principal signaller. Moreover, there were important differences in scent-marking behaviour between individuals, both in terms of marking frequency, and the social context of scent-marking (i.e. the identity of neighbours present at the time of scent-marking, and the response to deposited marks; see **Q1**–**Q2** in **Chapter 1**, section I.4.2). As detailed in **Chapter 2**, section II.4.1.1, reproduction in callitrichids is usually monopolized by the dominant, polyandrous female; while subordinate females typically show a suppression of ovulation, and act as helpers in the rearing of the young instead of investing into their own reproduction (Beehner and Lu, 2013; Savage, Ziegler and Snowdon, 1988; Snowdon et al., 2006; Ziegler and Sousa, 2002). Nevertheless, the strictness of this cooperative breeding model seems to vary between species and/or populations, as some authors have reported occurrences of multiple breeding females in the same group (reviewed in Smith et al., 2001a). Hence different reproductive strategies may be used by subordinate female callitrichids, following diverse levels of female intrasexual competition (Arruda et al., 2005; Heymann, 2006a; Lazaro-Perea, 2001). Moreover, as scent-marking plays a role in intersexual mate choice and advertisement of ovulation (Abbott et al., 1998; Smith et al., 2001b), the differences observed at the level of sex and the individual may indicate differences in female reproductive state across groups at the time of the study. In addition, although there was no difference in the chemical richness of the scent samples collected at the levels of sex, reproductive status, nor between individual female tamarins, their chemical diversity varied (see **Q6** & **Q9** in **Chapter 1**, section I.4.2). Similarly, differences have been reported in other primates (e.g. in ring-tailed lemur, *Lemur catta*, Boulet et al., 2010; Charpentier, Boulet and Drea, 2008; Greene and Drea, 2014; and

mandrills, Setchell et al., 2010; Vaglio et al., 2016). Hence, in callitrichids at least, scent-marking frequency does not appear to relate directly to the complexity of deposited scents; instead, both aspects of chemosignalling may be under differing influences of socio-sexual and environmental contexts (Greene and Drea, 2014).

V.1.1.3. Variation in scent-marking characteristics

As seen in **Chapter 2**, section II.4.1.2, captive callitrichid scent-marking behaviour may take various forms, thus contributing to the complexity of the signals conveyed via olfactory communication (see **Q3** in **Chapter 1**, section I.4.2). Notably, glandular secretions were sometimes mixed with voided urine. Voided urine can contain chemosignals in primates (Colquhoun, 2011; DelBarco-Trillo et al., 2012, 2013; Laska and Hudson, 1995; Palagi, Dapporto and Borgognini Tarli, 2005). In addition, silvery marmosets often associated scent-marking with tree-gouging, a common behaviour in marmosets (Rylands and Mittermeier, 2013), which may indicate a role for scent-marking behaviour in food resource signalling. Moreover, emperor and cotton-top tamarins primarily used their anogenital scent-gland for scent-marking, as was found in other tamarins (Heymann, 2001); while silvery marmosets mostly used their suprapubic gland, as shown in other marmosets (Rylands, 1984, 1990). Scent-marking duration was also variable, mainly of short and medium duration (i.e. <6 sec). Furthermore, **Chapter 3** established that scent-gland secretions, scent-marks and urine differed in their chemical composition (see **Q5** in **Chapter 1**, section I.4.2); and **Chapter 4** revealed chemical differences between scent-gland types (see **Q7** & **Q9** in **Chapter 1**, section I.4.2). Therefore, by adapting the relative amount of fluids deposited during scent-marking, and scent-gland use, callitrichids may be able to vary the chemosignals conveyed. Additionally, if scent-marking constitutes a visual signal as well as an olfactory one, as suggested in the ring-tailed lemur (Kappeler, 1998; Palagi and Norscia, 2009), varying scent-mark deposition may yield a variety of immediate visual signals able to be conveyed to conspecifics (Johnstone, 1996).

I also found temporal and spatial differences in scent-marking behaviour in captive callitrichids (see **Q4** in **Chapter 1**, section I.4.2).

Scent-marking activity was generally higher in the afternoon than in the morning for the two tamarin species. There also seemed to be a preference for certain enclosure areas amongst those available at each site, and for horizontal and inclined substrates. Spatiotemporal variation in scent-marking activity was similarly observed in other tamarin species (e.g. in golden lion tamarins, *Leontopithecus rosalia*, Miller, Laszlo and Dietz, 2003; moustached and Geoffroy's saddleback tamarins, Smith, 1997), and support the hypothesis that scent-marking is associated with orientation and food resource marking (Heymann, 2006a).

V.1.2. Identity of putative semiochemicals from captive and wild callitrichids

In **Chapter 3**, section III.3.3, and **Chapter 4**, sections IV.3.1.2 and IV.3.2.2, I identified a number of putative semiochemicals from the scent samples of captive silvery marmosets, emperor tamarins and cotton-top tamarins, and of wild emperor tamarins and saddleback tamarins, respectively (see **Q6**–**Q8** in **Chapter 1**, section I.4.2). These compounds of interest differed between species, groups, individuals, and at the level of sex and reproductive status, potentially constituting a first step in deciphering the role of chemosignals in these species. In addition, I revealed differences in the chemical composition of the three types of scent-gland (i.e. anogenital, suprapubic and sternal) in the two wild tamarin species (see **Chapter 4**, section IV.4.1). Chemical differences have similarly been found between labial, scrotal and brachial scent-glands of ring-tailed lemurs (Scordato, Dubay and Drea, 2007), and between subcaudal and pectoral scent-glands of owl monkeys (Spence-Aizenberg et al., 2018).

Some of the compounds identified as putative callitrichid semiochemicals have not been mentioned in any of the 92 publications reviewed in **Table V.1**. This was the case of pentan-2-ol, 3-hydroxybutan-2-one, 3-methylbutan-1-ol, methylcycloheptanone, 2-butoxyethanol, anisole, 3,7-dimethyloctan-3-ol, *trans*-1-methyl-4-(1-methylethyl)cyclohexanol, ethyl oct-3-enoate, cyclodecane, 3-methyltridecane, butyl 2-methylpent-4-enoate, ethyl dec-3-enoate, diethylene glycol dibutyl ester, myristicin, and 2-methyldecylpropanoate. Therefore, these compounds may be unique

semiochemicals of scent-gland secretions, scent-marks, and/or urine of one or several of the four callitrichid species studied, which could constitute an interesting novel addition to our current knowledge of mammalian semiochemistry. Nevertheless, a larger sample size and more advanced chemical analyses will be needed in order to help ensure that these compounds ***i.*** were assigned the correct identity by the mass spectral library, and ***ii.*** were not of exogenous origin.

Most of the compounds of interest identified in the present study have been mentioned in the existing literature on mammalian semiochemistry, in primates as well as rodents, lagomorphs, carnivores, artiodactyls, perissodactyls, marsupials, and elephants (**Table V.1**). Notably, a number of compounds were common, retrieved from the diverse types of sample analysed in my study, as well as from scent-gland secretions, scent-marks, skin swabs, urine, and/or faeces of diverse mammalian taxa. This was the case of butan-1-ol, hexanal, 2-methylpropanoic acid, heptanal, benzaldehyde, 1-octen-3-ol, acetophenone, p-cresol, 4-methoxybenzaldehyde, and dodecanoic acid. Nevertheless, these compounds are likely to serve different functions in different species, and/or may be semiochemicals only in some species.

In addition, in **Chapter 4** I compared the chemical composition of wild and captive emperor tamarin scent-gland swab samples, using identical analytical methods (see **Q8** in **Chapter 1**, section I.4.2). Butan-1-ol, 1-octen-3-ol, benzaldehyde, acetophenone, a branched C8 carboxylic acid, 4-methoxybenzaldehyde, and cyclododecane, were common to the two sets of samples; yet most compounds were not shared. This result indicates that captivity likely modifies the chemical composition of emperor tamarin scent-gland secretions, although not entirely. Indeed, diet, husbandry procedures (e.g. feeding and enrichment routines), climate, and illumination in a captive environment can influence an animal's bacterial environment and hence cause changes in the chemicals produced and used as signals (Drea et al., 2013; Kwak et al., 2008; Rudie, 2015).

I further compared the use of the new generation Torion® portable gas chromatography-mass spectrometry (GC-MS) device with that of a conventional bench-top GC-MS, for the analysis of wild tamarin headspace samples (see **Q11**–**Q12** in **Chapter 1**, section I.4.2). The number of volatile

chemicals retrieved using the Torion® was lower than with the laboratory bench-top instrument. This indicates that this portable device was probably a less suitable technique than the conventional laboratory-based techniques for the analysis of the complex mixtures of compounds that constitute tamarin chemosignals. Nevertheless, as detailed in **Chapter 4**, section IV.4.3, a number of methodological aspects can readily be optimized to ensure greater success in future use of the Torion® GC-MS, which has great potential to enhance the study of animal chemosignalling in field conditions.

Finally, in **Chapter 3** I explored the temporal stability of the samples collected for my study (see **Q10** in **Chapter 1**, section I.4.2). I found that compounds are lost and transform over time inside the sample vials, reflecting the versatility of scent signals. Such natural decay of scent signals is likely to participate to the information transmitted, indicating past physical presence and physiological state of the signaller animal (Müller-Schwarze, 2006). My experience also showed that sampling the headspace above the sample multiple times has more deleterious effect on the quality of the sample than storage at room temperature (see **Chapter 3**, sections III.3.4 and II.4.3). Overall, these results concur with the standard recommendation in this field, to store samples frozen before analysis and to extract each sample only once, in order to ensure optimum results (Apps, Weldon and Kramer, 2015; Drea et al., 2013).

Table V.1. Review of the compounds of interest identified in the present study, in the existing literature on mammalian semiochemicals, compiled from 92 publications from 1988–2019. Colour shading indicates primates, rodents and lagomorphs, carnivores, artiodactyls, and other taxa. SG= scent-gland; SM= deposited scent-mark; U= urine; F= faeces. References are indicated by numbers in super-script; ♦= present results from captive study (**Chapter 3**); †= present results from wild samples analysed *in situ*, and ✶= from wild samples analysed in the laboratory (**Chapter 4**).

Compound	Scent-glands / deposited scent-marks	Skin / body region	Urine / faeces
Butan-1-ol	Emperor tamarin, *Saguinus imperator subgrisescens* (all SG✶; suprapubic SG, SM♦)	Emperor tamarin (skin)✶	Brown rat, *Rattus norvegicus* (U)[4]
	Silvery marmoset, *Mico argentatus* (suprapubic SG)♦	Human, *Homo sapiens sapiens* (skin)[2,3]	Siberian tiger, *Panthera tigris altaica* (U)[5]
	Cotton-top tamarin, *Saguinus oedipus* (SM)♦		White-tailed deer, *Odocoileus virginianus* (U)[6]
	Owl monkey, *Aotus azarae* (subcaudal SG)[1]		
Methoxypropan-2-ol	Emperor tamarin (suprapubic SG)♦	Human (skin)[3]	House mouse, *Mus musculus domesticus* (U)[7]
	Silvery marmoset (suprapubic SG)♦	House mouse, (body)[7]	
Pentan-2-one	Emperor tamarin (SM)♦	Human (skin)[8]	Emperor tamarin (U)♦
	Cotton-top tamarin (SM)♦	House mouse (body)[7]	Brown lemurs, *Eulemur* spp. (U)[9]
	Owl monkey, *Aotus nancymaae* (subcaudal SG)[1]		Dwarf hamsters, *Phodopus* spp. (U)[10]
			House mouse (U)[7]
			Binturong, *Arctictis binturong* (U)[11]
			Lion, *Panthera leo* (U)[12,13]
			Cheetah, *Acinonyx jubatus* (U)[14]
			White-tailed deer (U)[6]
			African elephant, *Loxodonta africana* (U)[15]
Pentan-2-ol	Emperor tamarin (SM)♦		Emperor tamarin (U)♦
	Cotton-top tamarin (SM)♦		
	White-faced saki monkey, *Pithecia pithecia* (SM)♦		
3-hydroxybutan-2-one	Emperor tamarin (anogenital SG)✶		

Table V.1. Continued (1/14).

Compound	Scent-glands / deposited scent-marks	Skin / body region	Urine / faeces
3-methylbutan-1-ol	Emperor tamarin (anogenital SG)⋆		
Propane-1,2-diol	Emperor tamarin (suprapubic SG, SM)◆		African wild dog, *Lycaon pictus* (U, F)[16]
	Silvery marmoset (suprapubic SG)◆		Black-backed jackal, *Canis mesomelas* (F)[16]
Hexanal	Emperor tamarin (suprapubic SG, SM)◆	Common marmoset, *Callithrix jacchus* (body)[26]	Emperor tamarin (U)◆
	Cotton-top tamarin (SM)◆	Ring-tailed lemur, *Lemur catta* (tail)[17]	Cotton-top tamarin (U)◆
	Silvery marmoset (suprapubic SG)◆	Human (skin[3,27], para-axillary region[28])	Black-headed spider monkey, *Ateles fusciceps* (U)◆
	White-faced saki monkey (SM)◆	Waterbuck, *Kobus defassa* (body)[29]	House mouse (U)[7]
	Ring-tailed lemur (brachial SG)[17]		Ferret, *Mustela furo* (U)[30]
	Red-ruffed lemur, *Varecia rubra* (anogenital SG)[18]		Siberian tiger (U)[5]
	Mandrill, *Mandrillus sphinx* (sternal SG)[19]		Bengal tiger, *Panthera tigris tigris* (U)[31]
	Dwarf hamster (cheek SG)[20]		Lion (U)[12]
	Iberian wolf, *Canis lupus signatus* (anal SG)[21]		Cheetah (U)[14]
	Domestic dog, *Canis lupus familiaris* (anal SG)[16]		African wild dog (U, F)[16]
	Red hartebeest, *Alcephalus buselaphus caama* (interdigital SG)[22]		Eurasian otter, *Lutra lutra* (F)[32]
	Suni, *Neotragus moschatus* (preorbital SG)[23]		
	Sika deer, *Cervus nippon* (metatarsal SG)[24]		
	Koala, *Phascolarctos cinereus* (sternal SG)[25]		
2-Methylpropanoic acid (1/2)	Emperor tamarin (SM)◆		Emperor tamarin (U)◆
	Mandrill (sternal SG)[19,33]		Brown rat (F)[38]
	Chimpanzee, *Pan troglodytes* (vaginal secretions)[34]		African wild dog (U, F)[16]
	Dwarf hamster (cheek SG)[20]		Iberian wolf (F)[21]

Table V.1. Continued (2/14).

Compound	Scent-glands / deposited scent-marks	Skin / body region	Urine / faeces
2-Methylpropanoic acid (2/2)	Giant panda, *Ailuropoda melanoleuca* (SM)[35]		Black-backed jackal (F)[16]
	African wild dog (anal SG)[16]		Domestic dog (U)[16]
	Iberian wolf (anal SG)[21]		Eurasian otter (F)[32]
	Black-backed jackal, *Canis mesomelas* (anal SG)[16]		
	Wolverine, *Gulo gulo* (anal SG)[36]		
	Small Indian mongoose, *Herpestes auropunctatus* (anal SG)[37]		
	Coyote, *Canis latrans* (anal SG)[16]		
	Domestic dog (anal SG)[16]		
	Red fox, *Vulpes vulpes* (anal SG)[16]		
	Koala (sternal SG)[25]		
Furfural	Emperor tamarin (SM)◆	Bengal tiger (head)[40]	Siberian tiger (U)[5]
	Silvery marmoset (suprapubic SG)◆	Lion (head)[40]	African wild dog (U)[16]
	Common marmoset (SM)[39]	Leopard, *Panthera pardus* (head)[40]	
		Puma, *Puma concolor* (head)[40]	
3-Methylbutanoic acid (1/2)	Emperor tamarin (suprapubic SG, SM)◆	2-methylbutanoic acid: House mouse (body)[7]	2-methylbutanoic acid: House mouse (U)[7]
	White-faced saki monkey (SM)◆		2-methylbutanoic acid: African wild dog (U, F)[16]
	2-methylbutanoic acid: Dwarf hamster (cheek SG)[20]		2-methylbutanoic acid: Iberian wolf (F)[20]
	2-methylbutanoic acid: Iberian wolf (anal SG)[21]		
	2-methylbutanoic acid: Coyote (anal SG)[16]		
	2-methylbutanoic acid: Domestic dog (anal SG)[16]		
	2-methylbutanoic acid: Red fox (anal SG)[16]		
	2-methylbutanoic acid: Wolverine (anal SG)[36]		

Table V.1. Continued (3/14).

Compound	Scent-glands / deposited scent-marks	Skin / body region	Urine / faeces
3-Methylbutanoic acid (2/2)	2-methylbutanoic acid: Giant panda (SM)[35]		
	2-methylbutanoic acid: Koala (sternal SG)[25]		
2-Furanmethanol	Emperor tamarin (suprapubic SG, SM)◆	Human (skin[2], para-axillary region[28])	Cotton-top tamarin (U)◆
	Silvery marmoset (suprapubic SG)◆	Leopard (head)[40]	Domestic dog (U)[16]
	Cotton-top tamarin (SM)◆	Puma (head)[40]	Bengal tiger (U)[31]
		Bengal tiger (head)[40]	African wild dog (U, F)[16]
			Black-backed jackal (F)[16]
Cyclopent-2-en-1,4-dione	Emperor tamarin (SM)◆		
	Cotton-top tamarin (SM)◆		
	Common marmoset (SM)[39]		
1,2-Dimethylbenzene	Cotton-top tamarin (SM)◆		1,4-dimethylbenzene: House mouse (U)[7]
	White-faced saki monkey (SM)◆		Lion (U)[12]
	1,3-dimethylbenzene: European rabbit, *Oryctolagus cuniculus* (chin SG)[41]		Iberian wolf (F)[21]
	Giant panda (SM)[35]		
Methyl hexanoate	Weddell's saddleback tamarin, *Leontocebus weddelli* (all SG)†	Weddell's saddleback tamarin (skin)†	Iberian wolf (F)[21]
		Human (para-axillary region)[28]	
Methyl-cycloheptanone	Emperor tamarin (suprapubic SG, SM)◆		Cotton-top tamarin (U)◆
	Silvery marmoset (suprapubic SG)◆		
	Cotton-top tamarin (SM)◆		
Heptanal (1/2)	Emperor tamarin (SM)◆	Human (skin[3,8], para-axillary region[28])	Emperor tamarin (U)◆
	Cotton-top tamarin (SM)◆	House mouse (body)[7]	Cotton-top tamarin (U)◆
	Silvery marmoset (suprapubic SG)◆	Bengal tiger (head)[40]	Black-headed spider monkey (U)◆

Table V.1. Continued (4/14).

Compound	Scent-glands / deposited scent-marks	Skin / body region	Urine / faeces
Heptanal (2/2)	Mandrill (sternal SG)[19]	Lion (head)[40]	Dwarf hamster (U)[10]
	Red hartebeest (interdigital SG)[22]	Leopard (head)[40]	House mouse (U)[7]
	Suni (preorbital SG)[23]	Puma (head)[40]	Bengal tiger (U)[31]
	Sika deer (metatarsal SG)[24]	Reticulated giraffe, *Giraffa camelopardalis* (body)[42]	Lion (U)[12]
	Koala (sternal SG)[25]	African buffalo, *Syncerus caffer* (body)[29]	Eurasian otter (F)[32]
		Waterbuck (body)[29]	
2-butoxyethanol	Emperor tamarin (suprapubic, sternal SG)⋆	Emperor tamarin (skin)⋆	
Branched C9 alcohol	Emperor tamarin (all SG)⋆	Emperor tamarin (skin) ⋆	6-methyloctan-2-ol : Dwarf hamsters (U)[10]
2,5-Dimethylpyrazine	Silvery marmoset (suprapubic SG)◆	Common marmoset (body)[26]	House mouse (U)[43]
	Common marmoset (SM)[39]		Tree shrew, *Tupaia belangeri* (U)[38]
			Pine vole, *Microtus pinetorum* (U)[44]
			Dwarf hamsters (U)[10]
			Ferret (U)[30]
			Maned wolf, *Chrysocyon brachyurus* (U)[45]
			Lion (U)[13]
			Siberian tiger (U)[5]
			African wild dog (F)[16]
Anisole	Emperor tamarin (SM)◆		Emperor tamarin (U)◆
	Cotton-top tamarin (SM)◆		Cotton-top tamarin (U)◆
Benzaldehyde (1/2)	Emperor tamarin (anogenital SG†,⋆; suprapubic SG, SM◆)	Common marmoset (body)[26]	Emperor tamarin (U)◆
	Weddell's saddleback tamarin (anogenital SG)†	Human (skin[2,8,27], para-axillary region[28])	Cotton-top tamarin (U)◆
	Silvery marmoset (suprapubic SG)◆	House mouse (body)[7]	Black-headed spider monkey (U)◆

Table V.1. Continued (5/14).

Compound	Scent-glands / deposited scent-marks	Skin / body region	Urine / faeces
Benzaldehyde (2/2)	Cotton-top tamarin (SM)◆	Bengal tiger (head)[40]	Brown lemurs (U)[9]
	White-faced saki monkey (SM)◆	Reticulated giraffe (body)[42]	Pine vole (U)[44]
	Common marmoset (SM)[39]		Dwarf hamsters (U)[10]
	Owl monkey (subcaudal SG)[1]		House mouse (U)[7]
	Red-ruffed lemur (anogenital SG)[18]		Brown rat (U)[4]
	Mandrill (sternal SG)[19]		Giant panda (U)[47]
	European rabbit (chin SG)[41]		Bengal tiger (U)[31]
	Crested porcupine, *Hystrix cristata* (perianal SG)[46]		Siberian tiger (U)[5]
	Giant panda (SM)[35]		Lion (U)[12]
	African wild dog (anal SG)[16]		Cheetah (U)[14]
	Ferret (anal SG)[30]		Binturong (U)[11]
	Black-backed jackal (anal SG)[16]		Red fox (U)[16]
	Iberian wolf (anal SG)[21]		African wild dog (U, F)[16]
	Wolverine (anal SG)[36]		Ferret (U)[30]
			Black-backed jackal (F)[16]
			Iberian wolf (F)[21]
			Eurasian otter (F)[32]
1,3,5-Trimethylbenzene	Cotton-top tamarin (SM)◆		Eurasian otter (F)[32]
	Unknown trimethylbenzene: Common marmoset (SM)[39]		
1-Octen-3-ol (1/2)	Emperor tamarin (SM◆, suprapubic SG⋆)	Emperor tamarin (skin)⋆	Cotton-top tamarin (U)◆
	Cotton-top tamarin (SM)◆	Human (skin)[2]	Black-headed spider monkey (U)◆
	Silvery marmoset (suprapubic SG)◆		African wild dog (F)[16]
	African wild dog (anal SG)[16]		Eurasian otter (F)[32]
	Iberian wolf (anal SG)[21]		

Table V.1. Continued (6/14).

Compound	Scent-glands / deposited scent-marks	Skin / body region	Urine / faeces
1-Octen-3-ol (2/2)	Black-backed jackal (anal SG)[16]		
	Wolverine (anal SG)[36]		
Branched C12 alkane	Emperor tamarin (all SG)⋆	Emperor tamarin (skin)⋆	
	4-methylundecane: European rabbit (chin SG)[41]		
	2-methylundecane: Red hartebeest (interdigital SG)[22]		
6-Methyl-5-hepten-2-one	Emperor tamarin (SM)◆	Common marmoset (body)[26]	House mouse (U)[7]
	Silvery marmoset (suprapubic SG)◆	Human (skin[2,8,27], para-axillary region[28])	Dwarf hamsters (U)[10]
	Ferret (anal SG)[30]	House mouse (body)[7]	Ferret (U)[30]
		Bengal tiger (head)[40]	Maned wolf (U)[45]
		Lion (head)[40]	Red fox (U)[38]
		Leopard (head)[40]	White-tailed deer (U)[6]
		Puma (head)[40]	
		Waterbuck (body)[29]	
1,2,3- or 1,2,4-Trimethylbenzene	Cotton-top tamarin (SM)◆		Cotton-top tamarin (U)◆
	1,2,4-trimethylbenzene: European rabbit (chin SG)[41]		
Ethyl hexanoate	Emperor tamarin (sternal SG)⋆	Human (skin)[3]	
	Weddell's saddleback tamarin (anogenital SG)†		
Benzyl alcohol	Emperor tamarin (suprapubic SG, SM)◆	Human (para-axillary region)[28]	Emperor tamarin (U)◆
	Silvery marmoset (suprapubic SG)◆		Siberian tiger (U)[5]
	Black-backed jackal (anal SG)[16]		African wild dog (U, F)[16]
	Wolverine (anal SG)[36]		
Benzene acetaldehyde (1/2)	Emperor tamarin (suprapubic SG, SM)◆		Emperor tamarin (U)◆
	Silvery marmoset (suprapubic SG)◆		Cotton-top tamarin (U)◆

Table V.1. Continued (7/14).

Compound	Scent-glands / deposited scent-marks	Skin / body region	Urine / faeces
Benzene acetaldehyde (2/2)	Cotton-top tamarin (SM)◆		Black-headed spider monkey (U)◆
	Giant panda (SM)[35]		
Acetophenone	Emperor tamarin (suprapubic SG, SM◆; sternal SG⋆)	Emperor tamarin (skin)⋆	Emperor tamarin (U)◆
	Weddell's saddleback tamarin (sternal SG)†	Common marmoset (body)[26]	Cotton-top tamarin (U)◆
	Silvery marmoset (suprapubic SG)◆	Human (skin)[2]	Brown lemurs (U)[9]
	Cotton-top tamarin (SM)◆	Bengal tiger (head)[40]	House mouse (U)[43]
	North American beaver, *Castor canadensis* (anal SG)[38]	Lion (head)[40]	Dwarf hamsters (U)[10]
	African wild dog (anal SG)[16]	Leopard (head)[40]	Brown rat (U)[4]
	Ferret (anal SG)[30]	Puma (head)[40]	Bengal tiger (U)[31]
			Ferret (U)[30]
			Red fox (U)[38]
			Lion (U)[13]
			Cheetah (U)[14]
			Maned wolf (U)[45]
			Coyote (U)[16]
			Domestic dog (U)[16]
			Black-backed jackal (U, F)[16]
			African wild dog (U, F)[16]
			Iberian wolf (F)[21]
			African elephant (U)[15]
p-Cresol (1/2)	Emperor tamarin (suprapubic SG, SM)◆	Human (skin)[2]	Emperor tamarin (U)◆
	Silvery marmoset (suprapubic SG)◆	Reticulated giraffe (body)[42]	Cotton-top tamarin (U)◆
	Cotton-top tamarin (SM)◆		Black-headed spider monkey (U)◆
	Common marmoset (SM)[39]		Brown lemurs (U)[9]

Table V.1. Continued (8/14).

Compound	Scent-glands / deposited scent-marks	Skin / body region	Urine / faeces
p-Cresol (2/2)	Red-ruffed lemur (anogenital SG)[18]		Brown rat (U)[38]
	Mandrill (sternal SG)[33]		Pine vole (U)[44]
	Giant panda (SM)[35]		Lion (U)[13]
	Small Indian mongoose (anal SG)[37]		Siberian tiger (U)[5]
			African wild dog (U, F)[16]
			Iberian wolf (F)[21]
			Black-backed jackal (F)[16]
			Moose, *Alces alces* (U)[48]
			African elephant (U)[15]
			Horse, *Equus ferus* (U)[38]
Branched C15 alkane	Emperor tamarin (anogenital SG)†		
	2-methyltetradecane: Suni (preorbital SG)[23]		
p-Cymene	Cotton-top tamarin (SM)◆	Human (skin)[2,8]	Black-headed spider monkey (U)◆
	Giant panda (SM)[35]	Bengal tiger (head)[40]	Pine vole (U)[44]
	Koala (sternal SG)[25]	Lion (head)[40]	
		Leopard (head)[40]	
		Puma (head)[40]	
2-Methoxyphenol	Emperor tamarin (SM)◆	African buffalo (body)[29]	Emperor tamarin (U)◆
	Cotton-top tamarin (SM)◆	Waterbuck (body)[29]	Cotton-top tamarin (U)◆
		Zebu, *Bos taurus indicus* (body)[29]	Black-headed spider monkey (U)◆
			Brown rat (U)[4]
3,7-Dimethyloctan-3-ol	Emperor tamarin (suprapubic SG, SM)◆		
	Silvery marmoset (suprapubic SG)◆		
	Cotton-top tamarin (SM)◆		

Table V.1. Continued (9/14).

Compound	Scent-glands / deposited scent-marks	Skin / body region	Urine / faeces
Methyl octanoate	Emperor tamarin (SM)◆	Human (para-axillary region)[28]	
	Cotton-top tamarin (SM)◆		
Dimethyl pentanedioate	Emperor tamarin (SM)◆		Bengal tiger (U)[31]
	Cotton-top tamarin (SM)◆		
Branched C7 carboxylic acid	Emperor tamarin (sternal SG)⋆	2-methylhexanoic acid: Human (skin)[49]	
	2-methylhexanoic acid: Wolverine (anal SG)[36]		
	2-methylhexanoic acid: Small Indian mongoose (anal SG)[37]		
trans-1-Methyl-4-(1-methylethyl)-cyclohexanol	Emperor tamarin (SM)◆		Emperor tamarin (U)◆
	Silvery marmoset (suprapubic SG)◆		
1-Nonanol	Emperor tamarin (SM)◆		Emperor tamarin (U)◆
	Cotton-top tamarin (SM)◆		Bengal tiger (U)[31]
Branched C8 carboxylic acid	Emperor tamarin (suprapubic SG)◆,⋆	2-methylheptanoic acid: Human (skin)[49]	Emperor tamarin (U)◆
	Silvery marmoset (suprapubic SG)◆		Cotton-top tamarin (U)◆
	6-methylheptanoic acid: Red hartebeest (interdigital SG)[22]		
Ethyl oct-3-enoate	Emperor tamarin (sternal SG)⋆		
Ethyl octanoate	Emperor tamarin (all SG)⋆		
	Meerkat, *Suricata suricatta* (anal SG)[50]		
Dimethyl hexanedioate	Emperor tamarin (SM)◆	Human (para-axillary region)[28]	Bengal tiger (U)[31]
	Cotton-top tamarin (SM)◆		
4-Methoxy-benzaldehyde (1/2)	Emperor tamarin (anogenital SG⋆,†, suprapubic SG†,◆, SM◆)		Emperor tamarin (U)◆
	Silvery marmoset (suprapubic SG)◆		Cotton-top tamarin (U)◆

Table V.1. Continued (10/14).

Compound	Scent-glands / deposited scent-marks	Skin / body region	Urine / faeces
4-Methoxy-benzaldehyde (2/2)	Cotton-top tamarin (SM)◆,[51]		Black-headed spider monkey (U)◆
	Common marmoset (SM)[39]		
	White-faced saki monkey (SM)◆		
Cyclodecane	Emperor tamarin (suprapubic SG, SM)◆		Emperor tamarin (U)◆
	Silvery marmoset (suprapubic SG)◆		Cotton-top tamarin (U)◆
	Cotton-top tamarin (SM)◆		
Branched C13 alkane	Emperor tamarin (SM)◆		2,8-dimethylundecane: Eurasian otter (F)[32]
	Cotton-top tamarin (SM)◆		
	Silvery marmoset (suprapubic SG)◆		
	4-methyl- and 6-methyl-dodecane, 3,6-dimethylundecane: Mandrill (sternal SG)[33]		
Eugenol	Emperor tamarin (SM)◆	Human (skin)[52]	Cotton-top tamarin (U)◆
	Cotton-top tamarin (SM)◆		
3-Methyltridecane	Emperor tamarin (SM)◆		
Butyl 2-methylpent-4-enoate	Emperor tamarin (sternal SG)⋆		
Decanoic acid	Emperor tamarin (sternal SG)⋆	Human (skin)[49]	Bengal tiger (U)[31]
	Crested porcupine (perianal SG)[46]	Bengal tiger (head)[40]	African wild dog (U)[16]
	Brown bear, *Ursus arctos* (pedal SG)[53]	Lion (head)[40]	
	Wolverine (anal SG)[36]	Leopard (head)[40]	
	Sika deer (metatarsal SG)[24]	Puma (head)[40]	
		Waterbuck (body)[29]	

Table V.1. Continued (11/14).

Compound	Scent-glands / deposited scent-marks	Skin / body region	Urine / faeces
Ethyl dec-3-enoate	Emperor tamarin (sternal SG)⋆		
Ethyl decanoate	Emperor tamarin (anogenital, suprapubic, sternal SG)⋆		Bengal tiger (U)[31]
	Meerkat (anal SG)[50]		
Dodecanal or tridecanal	Emperor tamarin (SM)◆	dodecanal: Human (skin)[2,27]; tridecanal (skin)[52]	Emperor tamarin (U)◆
	Cotton-top tamarin (SM)◆	do- and tri-decanal: African buffalo (body)[29]	Cotton-top tamarin (U)◆
	Silvery marmoset (suprapubic SG)◆	do- and tri-decanal: Zebu (body)[29]	do- and tridecanal: African wild dog (U, F)[16]
	White-faced saki monkey (SM)◆		dodecanal: Lion (U)[13]
	do- and tri-decanal: Mandrill (sternal SG)[19]		dodecanal: Iberian wolf (F)[21]
	dodecanal: African wild dog (anal SG)[16]		dodecanal: Coyote (U)[16]
	do- and tri-decanal: Sika deer (metatarsal SG)[24]		dodecanal: Asian elephant, *Elephas maximus* (U)[54]
Diethylene glycol dibutyl ether	Emperor tamarin (suprapubic SG, SM)◆		Emperor tamarin (U)◆
	Silvery marmoset (suprapubic SG)◆		
Cyclododecane	Emperor tamarin (anogenital SG⋆, suprapubic SG, SM◆)		Emperor tamarin (U)◆
	Cotton-top tamarin (SM)◆		Cotton-top tamarin (U)◆
	Silvery marmoset (suprapubic SG)◆		Black-headed spider monkey (U)◆
	White-faced saki monkey (SM)◆		
	Ring-tailed lemur (anogenital SG)[55]		
	Coquerel's sifaka, *Propithecus coquereli* (anogenital SG)[55]		
Myristicin	Emperor tamarin (SM)◆		Emperor tamarin (U)◆
	Cotton-top tamarin (SM)◆		

Table V.1. Continued (12/14).

Compound	Scent-glands / deposited scent-marks	Skin / body region	Urine / faeces
2-Methyldecyl-propanoate	Cotton-top tamarin (SM)◆		Cotton-top tamarin (U)◆
Dodecanoic acid	Emperor tamarin (anogenital SG)⋆	Human (skin[2,49], para-axillary region[28])	African wild dog (U)[16]
	North American beaver (anal SG)[38]	Bengal tiger (head)[40]	Eurasian lynx (U)[58]
	Crested porcupine (perianal SG)[46]	Lion (head)[40]	Bengal tiger (U)[31]
	African wild dog (preputial SG)[16]	Leopard (head)[40]	Iberian wolf (F)[21]
	European badger, *Meles meles* (anal SG)[56]	Puma (head)[40]	
	Banded mongoose, *Mungos mungo* (anal SG)[57]		
	Meerkat (anal SG)[50]		
	Wolverine (anal SG)[36]		
	Sika deer (metatarsal SG)[24]		
Ethyl dodecanoate	Emperor tamarin (anogenital, sternal SG)⋆		
	Meerkat (anal SG)[50]		
Branched C16 alkane	Emperor tamarin (suprapubic SG, SM)◆		Emperor tamarin (U)◆
	Cotton-top tamarin (SM)◆		Cotton-top tamarin (U)◆
	Silvery marmoset (suprapubic SG)◆		3-methyl- and 7-methyl-pentadecane: Eurasian otter (F)[32]
	White-faced saki monkey (SM)◆		
	2-methyl- and 3-methyl-pentadecane: Mandrill (sternal SG)[19]		
Tetradecanal or pentadecanal (1/2)	Cotton-top tamarin (SM)◆	tetradecanal: Human (para-axillary region)[28]	pentadecanal: African wild dog (U, F)[16]
	tetradecanal: Mandrill (sternal SG)[19]	tetra- and penta-decanal: African buffalo (body)[29]	tetra- and penta-decanal: Iberian wolf (F)[21]
	pentadecanal: Crested porcupine (perianal SG)[46]	tetra- and penta-decanal: Zebu (body)[29]	
	tetra- and penta-decanal: Giant panda (anogenital SG)[59]		

Table V.1. Continued (13/14).

Compound	Scent-glands / deposited scent-marks	Skin / body region	Urine / faeces
Tetradecanal or pentadecanal (2/2)	tetra- and penta-decanal: Meerkat (anal SG)[50]		
	pentadecanal: African wild dog (anal SG)[16]		
	tetradecanal: Sika deer (metatarsal SG)[24]		
Hexadecan-1-ol	Weddell's saddleback tamarin (suprapubic SG)†	Rhesus macaque, *Macaca mulatta* (body)[62]	African wild dog (U, F)[16]
	Ring-tailed lemur (anogenital SG)[55]	Bengal tiger (head)[40]	Bengal tiger (U)[31]
	Coquerel's sifaka (anogenital SG)[55]	Lion (head)[40]	Iberian wolf (F)[21]
	House mouse (preputial SG)[60]	Leopard (head)[40]	
	Syrian golden hamster, *Mesocricetus auratus* (vaginal secretions)[61]	Puma (head)[40]	
	Crested porcupine (perianal SG)[46]		
	African wild dog (anal SG)[16]		
	Black-backed jackal (anal SG)[16]		
	Small dwarf mongoose, *Helogale parvula* (anal SG)[63]		
	Banded mongoose (anal SG)[57]		
	Meerkat (anal SG)[50]		
	Brown bear (pedal SG)[53]		
	Sika deer (metatarsal SG)[24]		
Heptadecane	Cotton-top tamarin (SM)◆	Human (para-axillary region)[28]	
	Silvery marmoset (suprapubic SG)◆		
	Red-ruffed lemur (anogenital SG)[18]		
	Mandrill (sternal SG)[19]		
	Giant panda (anogenital SG)[64]		
	Suni (preorbital SG)[23]		

Table V.1. Continued (14/14).

Compound	Scent-glands / deposited scent-marks	Skin / body region	Urine / faeces
Ethyl tetradecanoate	Emperor tamarin (anogenital SG)⋆	Human (skin)[52]	
	Meerkat (anal SG)[50]		
	Giant panda (anogenital SG)[59]		
Ethyl pentadecanoate	Emperor tamarin (anogenital, suprapubic SG)⋆	Human (skin)[52]	
	Meerkat (anal SG)[50]		
	Giant panda (anogenital SG)[59]		

1. Spence-Aizenberg et al., 2018; **2.** Gallagher et al., 2008; **3.** Meijerink et al., 2000; **4.** Osada, Kashiwayanagi and Izumi, 2009; **5.** Soso and Koziel, 2016; **6.** Miller et al., 1998; **7.** Röck et al., 2006; **8.** Mochalski et al., 2014; **9.** Delbarco-Trillo et al., 2011; **10.** Soini et al., 2005; **11.** Greene et al., 2016b; **12.** Andersen and Vulpius, 1999; **13.** Soso and Koziel, 2017; **14.** Burger et al., 2006; **15.** Goodwin et al., 2012; **16.** Apps, Mmualefe and McNutt, 2012; **17.** Knapp, Robson and Waterhouse, 2006; **18.** Janda et al., 2019; **19.** Vaglio et al., 2016; **20.** Burger et al., 2001; **21.** Martín, Barja and López, 2010; **22.** Reiter, Burger and Dry, 2003; **23.** Stander, Burger and Le Roux, 2002; **24.** Wood, 2003; **25.** Salamon and Davies, 1998; **26.** Kücklich et al., 2017; **27.** Logan et al., 2008; **28.** Curran et al., 2005; **29.** Gikonyo et al., 2002; **30.** Zhang et al., 2005; **31.** Burger et al., 2008; **32.** Kean, Chadwick and Müller, 2015; **33.** Setchell et al., 2010; **34.** Matsumoto-Oda et al., 2003; **35.** Hagey and Macdonald, 2003; **36.** Wood, Terwilliger and Copeland, 2005; **37.** Miyazaki et al., 2018; **38.** Apps, Weldon and Kramer, 2015; **39.** Smith et al., 2001b; **40.** Soini et al., 2012; **41.** Hayes, Richardson and Wyllie, 2002; **42.** Wood and Weldon, 2002; **43.** Novotny et al., 2007; **44.** Boyer et al., 1989; **45.** Jones, 2017; **46.** Massolo, Dani and Bella, 2009; **47.** Wilson et al., 2018; **48.** Whittle et al., 2000; **49.** Akin, 2005; **50.** Leclaire et al., 2017; **51.** Belcher et al., 1988; **52.** Penn et al., 2007; **53.** Sergiel et al., 2017; **54.** Rasmussen, Krishnamurthy and Sukumar, 2005; **55.** Hayes, Morelli and Wright, 2004; **56.** Buesching, Waterhouse and Macdonald, 2002a; **57.** Jordan et al., 2010; **58.** Vogt et al., 2016; **59.** Yuan et al., 2004; **60.** Zhang et al., 2007; **61.** Briand et al., 2004; **62.** Birkemeyer et al., 2016; **63.** Decker, Ringelberg and White, 1992; **64.** Zhang et al., 2008.

V.2. Limits of the study and recommendations for future research

V.2.1. Importance of conducting behavioural bioassays

As mentioned in **Chapter 1** (section I.2.1.2; **Table I.2**), behavioural bioassays, in which odours are experimentally presented to target animals, are able to inform on the proximate functions of a particular intra- or inter-specific olfactory signal (Haynes and Millar, 1998). For instance, Smith and co-workers (Smith et al., 1997; Smith and Abbott, 1998) showed evidence for behavioural discrimination between circumgenital odours of familiar and unfamiliar individuals, and of periovulatory and anovulatory female common marmosets, by performing a series of behavioural bioassays in laboratory-housed animals. Henkel et al. (2015) similarly demonstrated that semi-free range rhesus macaques, *Macaca mulatta*, are able to recognize group members via olfactory cues alone. Without testing the behavioural, physiological, and/or neuronal response of an animal to a scent signal, it is not possible to infer its exact function. Therefore, although my study indicates that scent-marking behaviour may have a function in mate choice, intraspecific competition, and regulation of reproduction in callitrichids, no definite answer can be given without first carrying out bioassays.

As well as informing us on potential species-specific functions of chemosignalling, bioassays may help the understanding of a species' biology and physiology, as our knowledge of the exact mechanisms of the olfaction sense is still limited (Alberts, 1992; Heymann, 2006b; Wackermannová, Pinc and Jebavý, 2016). For example, functional magnetic resonance imaging (fMRI) has been used to identify the brain areas involved in the sexual arousal of male common marmosets in immediate response to the presentation of female odours (Ferris et al., 2001; reviewed in Snowdon et al., 2006). Moreover, behavioural bioassays can inform us on species-specific differences in odour detection abilities. For instance, experimental research by Laska and co-workers (Eliasson, Hernandez Salazar and Laska, 2015; Laska et al., 2004, 2007; Laska and Hudson, 1995; Laska, Seibt and Weber, 2000; Laska, Wieser and Hernandez Salazar, 2005), based on behavioural bioassays on common squirrel monkeys, *Saimiri sciureus*, Geoffroy's spider monkeys, *Ateles*

geoffroyi, and pig-tailed macaques, *Macaca nemestrina*, is progressively filling the gap of knowledge of odour detection thresholds in primates.

V.2.2. Methodological challenges to the study of wild primate chemosignalling

As highlighted by Heymann (2006b), progress in semiochemistry research in the field of primate olfactory communication is highly dependent on future methodological advances. Unlike acoustic and visual signals, the properties of an olfactory signal cannot be easily measured and quantified, especially in field conditions. Nevertheless, rigorous recording and quantification of chemosignals will be essential if future research is to understand functional relationships. As sample collection and analytical techniques develop, hence providing guidelines for future studies, there is hope that the challenges of semiochemistry research in field conditions will progressively be overcome. Novel techniques are starting to be employed in this field for the study of large mammals and humans, such as electronic noses (e.g. lemur scent-gland secretion composition, Staples and Electronic Sensor Technology, n.d.; multiple applications to the screening of human diseases reviewed by Röck, Barsan and Weimar, 2008), thermal desorption (e.g. body odour of common marmosets, Kücklich et al., 2017; feathers of king penguins, *Aptenodytes patagonicus*, Gabirot et al., 2018; human foot odour, Stevens et al., 2015), and portable GC-MS devices (e.g. body odour of common marmosets, Kücklich et al., 2017; wild tamarin scent-gland secretions, present study). However, these techniques require further sophistication and optimization to be usable at most primate field study sites, where vegetation, humidity, as well as conditions of storage and transportation, are still a challenge.

V.2.3. Need for a more cross-disciplinary research

In their review of the chemical ecology of mammalian communication, Charpentier and co-workers (2012) draw attention to the errors commonly found in the mammalian semiochemistry literature over the past two decades, in chemical characterization of molecules, as well as interpretation of their roles as chemical mediators of communication. For

example, semiochemicals reported in the literature are often misnamed, and sometimes inorganic compounds, undoubtedly originating from contamination, are mistakenly provided as putative identity. In particular, primatologists in the field of chemical ecology and semiochemistry need to acquire a stronger expertise in chemistry in order to progress in the deciphering of primate chemosignalling, or seek collaboration with specialists in analytical biochemistry (Charpentier et al., 2012; Drea et al., 2013).

One markedly growing cross-disciplinary field of research is microbial ecology. As discussed in **Chapters 3**–**4**, commensal bacteria and other microorganisms contribute to the production of animal chemosignals (reviewed in Archie and Theis, 2011; and Ezenwa and Williams, 2014). A number of studies have started to combine mammalian semiochemistry with bacterial culture (e.g. analysis of the tarsal tufts of white-tailed deer, *Odocoileus virginianus*, Alexy et al., 2003; and urine of African elephants, *Loxodonta africana*, Goodwin et al., 2012), and microbiomic analyses, i.e. bacterial DNA or rRNA sequencing (e.g. analysis of the anal sac of spotted hyenas, Theis, Schmidt and Holekamp, 2012; Theis et al., 2013; the anal sac of wild meerkats, *Suricata suricatta*, Leclaire et al., 2017; and the human foot, Stevens et al., 2015). A better understanding of the interactions at play between bacteria communities and their animal hosts has the potential to enhance the study of both animal behaviour and semiochemistry (Archie and Theis, 2011; Leclaire et al., 2017).

V.2.4. When communication goes multimodal

One of the main criticisms made about studies of animal communication is that they often look at acoustic, visual, or olfactory signalling as a discrete modality. In fact, much, if not all, of primate signalling is multimodal, i.e. signals are simultaneously sent via several communication channels (Liebal et al., 2014). The message resulting from a single modality might fail to capture the full richness and complexity of the global signal (Partan, 2013; Semple & Higham 2013). It is thus ideal, when studying primate communication, to investigate signals through several modalities. For instance, Buesching et al. (1998) showed that female mouse lemurs, *Microcebus murinus*, a nocturnal primate, use a

combination of olfactory (i.e. scent-marking activity) and acoustic (i.e. mating trills) cues, resulting in a multimodal advertisement of their oestrus. Similarly, Partan and Marler (1999) demonstrated the bimodality of threat displays in rhesus macaques, which combine visual (i.e. the open-mouth facial expression) with acoustic cues (i.e. the "bark" vocalization); and Palagi and Norscia (2009) suggested that ring-tailed lemurs' olfactory communication combines olfactory cues (i.e. urine-marking) with visual signals (i.e. ostentatiously raised tail). However, experimental designs conjointly assessing two or more communication modalities can be understandably difficult to carry out, due to methodological constraints (Moreira, Pessoa and Sousa, 2013). Consequently, a general recommendation is to put individual results in the perspective of a more complete, multimodal approach (Dominy, Ross and Smith, 2004; Higham and Hebets, 2013; Liebal et al., 2014; Waller et al., 2013).

V.3. Applications of semiochemistry research

V.3.1. Application to conservation breeding programmes

Reproductive success can have important consequences for the conservation of rare mammal species. When a wild population becomes too small or too inbred, or in the case of captive animals kept in unnatural social conditions (e.g. in very small groups in the case of social species, in groups in the case of solitary animals, or in groups with unbalanced sex ratio), breeding success may decrease, sometimes to an alarming level (Swaisgood and Schulte, 2010). As noted in **Chapters 2**–**4**, olfactory signals are thought to be important regulators of mate choice, intrasexual competition and sexual receptivity in mammals (Wyatt, 2014a). Modern breeding programmes are starting to make use of natural reproductive cues to artificially enhance breeding success in these populations at risk (Lindburg and Fitch-Snyder, 1994; Swaisgood and Schulte, 2010). For instance, the work by Swaisgood and co-workers (Swaisgood, Lindburg and Zhou, 1999; Swaisgood et al., 2000; Swaisgood, Lindburg and Zhang, 2002; Swaisgood et al., 2004; White, Swaisgood and Zhang, 2003) on captive giant pandas, *Ailuropoda melanoleuca*, was able to increase the natural mating record at the Wolong breeding centre (China) to the highest

of any giant panda breeding facility, by taking into account the importance of chemosignalling in this species. Notably, their research revealed the releaser effect, i.e. behavioural effect, that panda odours have on the sexual receptiveness of individuals of the opposite sex, and successfully exploited this effect: they exposed periestrous females and their mate-to-be to each other's scents prior to the physical mating introduction. This initial olfactory familiarization resulted in a decreased aggression, and an increased sexual activity, between the male and the female panda once placed in the same enclosure (Swaisgood et al., 2004). Furthermore, Fisher, Swaisgood and Fitch-Snyder (2003a; b) used odour cues to experimentally manipulate mate choice in a small wild population of the threatened pygmy loris, *Nycticebus pygmaeus*: they artificially presented females with scent-marks from specific males chosen on the basis of optimal outbreeding. As shown in mice by Rich and Hurst (1999), and Gosling and Roberts (2001a), familiarity with a male's odour may be one of the proximate mechanisms by which females choose a mate, because the ability of the male to countermark his rivals' scent-marks can be seen as an honest signal of high mate quality (reviewed in Swaisgood and Schulte, 2010). Fisher et al.'s (2003a; b) experimental olfactory manipulation successfully resulted in a genetically optimal mating, which reinforced the status of the population of pygmy lorises. A similar experimental manipulation was conducted by Roberts and Gosling (2004) on female harvest mice, *Micromys minutus*, where increased familiarity of females with the scent of particular males enhanced mating compatibility between them, which improved the conservation of this species in the wild. Such approaches could very well be implemented to aid the conservation of wild populations of endangered callitrichids, such as golden lion tamarins in the highly fragmented Brazilian lowland Atlantic rainforest (Kierulff et al., 2012; Rylands and Mittermeier, 2013).

V.3.2. Application to captive husbandry and welfare

Knowledge of mammalian chemosignalling is not only beneficial to conservation breeding programmes of rare species; it can also be used to stimulate reproduction in agricultural species. The primer effect of male odours to stimulate female sexual receptivity, called the Whitten effect (Whitten, Bronson and Greenstein, 1968), and their ability to accelerate

sexual maturation in young females, called the male effect, or the Vandenbergh effect (Vandenbergh, 1969), has been long known in livestock (e.g. in pigs, *Sus scrofa*, Brooks and Cole, 1970; sheep, *Ovis aries*, Knight and Lynch, 1980; and cattle, *Bos taurus*, Zalesky et al., 1984). The economic benefits of using these natural primer effects to increase the onset of puberty and reduce postpartum anoestrus in domestic animals can notably serve as a management tool in tropical areas, where livestock production faces more challenges than in temperate climates (Rekwot et al., 2001). Such knowledge may also be employed to enhance breeding in zoo facilities (Dehnhard, 2011).

The relatively new concept of 'olfactory enrichment' in zoo environments, where olfactory stimuli are introduced to an animal's enclosure to enhance activity and welfare, is proving successful (Clark and King, 2008; Wells, 2009). For example, captive lions, *P. leo*, exposed to the faeces of their natural prey species such as zebras, *Equus quagga*, and gazelles, *Gazella* spp., showed increased activity (Schuett and Frase, 2001). In a zoo environment, the animals' diet, the choice of enclosure furnishing elements, and the frequency and mode of cleaning, all have a potentially high impact on the olfactory communication of zoo residents, which should be taken into account in captive husbandry procedures (Dehnhard, 2011; Jennings and Prescott, 2009; Surov and Maltsev, 2016). Further knowledge in this area still needs to be assembled from all the trials conducted by individual zoos and laboratories, in order to draw comprehensive guidelines in this respect for the welfare of captive animals (Clark and King, 2008), including non-human primates (Jennings and Prescott, 2009; Wormell et al., 2012).

Pheromonatherapy, a modern branch of veterinary medicine, makes use of chemical signals to relieve stress and reduce behavioural disorders in pets (Mills, Braem Dube and Zulch, 2013). In particular, two chemical products are currently commercially available for clinical use, synthetically replicated from naturally produced glandular compounds. The first product, the dog-appeasing pheromone (DAP), is originally derived from the region around the mammary gland of the domestic dog, *Canis lupus familiaris*. It is commercialized as a spray, a diffuser and an impregnated collar, and has been reported to have a calming effect on dogs, thus relieving diverse behavioural problems such as anxiety during travelling by car, and

adaptation to an unfamiliar environment such as a veterinary hospital or a new kennel (Mills, Braem Dube and Zulch, 2013). The second product, the feline facial fraction, is originally issued from the perioral and cheek regions of the domestic cat, *Felis catus*. A couple of these cat-specific fraction mixtures are currently commercialized as sprays and diffusers for the house, which have been shown to resolve problems of urine spraying (Mills, Redgate and Landsberg, 2011), and to reduce aggression towards unfamiliar people and other pets (Mills, Braem Dube and Zulch, 2013). Moreover, the use of feline facial fraction has successfully helped reducing stereotypical urine spraying and head rubbing in captive tigers, *Panthera tigris,* although interestingly it showed no effect on lions (Spielman, 2000). In addition, novel research on human psychology has suggested that states of stress, happiness, and friendliness, are likely to be conveyed by human body odours (de Groot et al., 2015; Gaby and Zayas, 2017); all the more personal information that could unconsciously be transmitted during social encounters. Such new knowledge of how olfactory communication contributes to the regulation of social interactions may prove useful in improving welfare in highly social zoo animals such as monkeys and apes (Clark and King, 2008).

Finally, as much as odours may attract or soothe receiver animals, they can also serve as a repellent. In particular, biological control research is interested in using kairomones, i.e. the chemical signals used in recognition between prey and predator, to deter invasive mammals from certain areas. For instance, Ferrero et al. (2011) isolated 2-phenylethylamine from the urine of various felids, and demonstrated its repelling effect on mice, *Mus musculus*, and rats, *Rattus* spp. The use of kairomones in pest control – a non-painful, non-toxic, and environmentally friendly method – constitutes a good alternative to the current approaches in place (Grau et al., 2019).

V.4. General conclusion

The present study constitutes an important contribution to the field of mammalian communication in directly comparing scent-marking behaviour and semiochemistry in multiple species of callitrichids. I found differences in scent-marking behaviour and semiochemistry of captive and

wild callitrichids at the levels of species, group, sex, reproductive status, and the individual. My results support the idea that chemosignalling plays an important role in the advertisement of identity, reproductive state/status and dominance in this taxon. Moreover, I showed that the social context, as well as spatiotemporal aspects of scent-marking deposition, influenced scent-marking activity, which suggests a complex pattern of individually tuned signals transmitted via olfactory communication. Further differences in the characteristics of scent-marking deposition revealed in this study, both at behavioural and chemical levels, may reflect variable strategies of communication to ensure that signals are conveyed to the intended receivers, which is especially relevant for sympatric species.

I was also able to identify a number of putative semiochemicals from the scent samples of captive and wild callitrichids, which contributes to the current knowledge of mammalian chemosignals. Notably, I presented results from the first use of the Torion® portable GC-MS for *in situ* analysis of wild tamarin scent-gland samples. This portable device yielded results of lower quality than the analysis of similar samples in the laboratory. I provided recommendations for future use of portable GC-MS for *in situ* analyses of mammalian chemosignals, which have considerable potential to enhance the field of semiochemistry in wild conditions. Moreover, I found differences in the chemical composition of tamarin scent-gland secretion samples between wild and captive conditions, which may indicate an effect of captivity on the chemicals produced. This contrast between captive and wild tamarin chemical communication constitutes a particularly novel aspect in the study of mammalian chemosignalling, applicable to conservation breeding programmes, captive husbandry and animal welfare.

End of word count.

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Appendices

Appendix A

Table A.1. List of review articles and books (in bold) included in **Fig. I.1**, on the exclusive or partial topic of mammalian semiochemistry, published between 1959–2016.

Karlson and Butenandt, 1959	Snowdon et al., 2006
Wilson and Bossert, 1963	**Wyatt, 2006** (2nd ed. 2014)
Epple and Lorenz, 1967	Ferkin and Pierce, 2007
Tembrock, 1968	Johansson and Jones, 2007
Gleason and Reynierse, 1969	Symonds and Elgar, 2008
Schultze-Westrum, 1969	Havlicek and Roberts, 2009
Mykytowycz, 1970	Swaney and Keverne, 2009
Wilson, 1970	Wyatt, 2009
Cheal and Sprott, 1971	Colquhoun, 2011
Thiessen, Owen and Lindzey, 1971	Dehnhard, 2011
Eisenberg and Kleiman, 1972	Smith et al., 2011
Epple, 1972	Charpentier et al., 2012
Estes, 1972	Roberts, 2012
Epple, 1974a; b	Apps, 2013
Steiner, 1974	Beehner and Lu, 2013
(Thiessen and Rice, 1976)	Charpentier et al., 2013
(Ritter, 1979)	Drea et al., 2013
(Epple, 1981)	Moreira, Pessoa and Sousa, 2013
Albone and Shirley, 1984	Petrulis, 2013
Brown and Macdonald, 1985	Semple and Higham, 2013
Epple, 1986	Stockley, Bottell and Hurst, 2013
Gosling and McKay, 1990	De Lacy Costello et al., 2014
Alberts, 1992	DelBarco-Trillo and Drea, 2014
Epple et al., 1993	Ezenwa and Williams, 2014
Penn and Potts, 1998	**Liebal et al., 2014**
Gosling and Roberts, 2001a; b	Schaal and Aïn, 2014
Heymann, 2003	Soso et al., 2014
Johnston, 2003	Wyatt, 2014b
Dominy, Ross and Smith, 2004	Apps, Weldon and Kramer, 2015
Smith and Bhatnagar, 2004	Baum and Cherry, 2015
Thom and Hurst, 2004	Corona and Lévy, 2015
Burger, 2005	Drea, 2015
Barton, 2006	Ferkin, 2015
Brennan and Kendrick, 2006	Lübke and Pause, 2015
Evans, 2006	Martín-Sánchez et al., 2015
Heymann, 2006a; b	Wyatt, 2015
Müller-Schwarze, 2006	Setchell, 2016
Mundy, 2006	Seyfarth and Cheney, 2016
Smith, 2006	Surov and Maltsev, 2016
Smith and Rossie, 2006	.

Appendix B

Table B.1. Weight matrix representing hourly scent-marking frequency given the identity of the nearest neighbour, for each individual silvery marmoset at TZ. Numbers in bold represent hourly scent-marking frequencies weighted for the proportion of time the pair spent in proximity, used to create the sociogram in **Fig. II.6**; numbers in brackets are the original hourly scent-marking frequencies.

Silvery marmosets at TZ		**Nearest neighbour**								
		Repro. ♀	Repro. ♂	Older subord. ♀	Subord. ♀	Subord. ♂	Juvenile ♀	Juvenile ♂	Infant	None
Signalling individual	Repro. ♀		**0.059** (0.050)	**0.281** (0.025)	**0.234** (0.050)	**0.000** (0.000)	**0.047** (0.025)	**0.140** (0.025)	**0.056** (0.025)	**0.000** (0.000)
	Repro. ♂	**0.912** (0.325)		**0.284** (0.025)	**0.594** (0.200)	**0.219** (0.125)	**0.852** (0.075)	**0.214** (0.050)	**0.190** (0.075)	**0.069** (0.075)
	Older subord. ♀	**0.000** (0.000)	**0.278** (0.025)		**0.000** (0.000)	**0.000** (0.000)	**0.000** (0.000)	**0.000** (0.000)	**0.000** (0.000)	**0.126** (0.100)
	Subord. ♀	**0.520** (0.150)	**0.629** (0.200)	**0.025** (0.025)		**0.000** (0.100)	**0.407** (0.075)	**0.000** (0.150)	**0.000** (0.000)	**0.916** (0.250)
	Subord. ♂	**0.267** (0.125)	**0.354** (0.075)	**0.481** (0.075)	**0.137** (0.025)		**0.091** (0.025)	**0.055** (0.025)	**0.274** (0.075)	**1.267** (0.275)
	Juvenile ♀	**0.000** (0.000)	**0.140** (0.025)	**0.000** (0.000)	**0.140** (0.025)	**0.000** (0.000)		**0.141** (0.100)	**0.000** (0.000)	**0.000** (0.000)
	Juvenile ♂	**0.229** (0.100)	**0.000** (0.050)	**0.357** (0.100)	**0.000** (0.000)	**0.028** (0.025)	**0.179** (0.125)		**0.056** (0.025)	**0.000** (0.000)

Table B.2. Weight matrix representing hourly scent-marking frequency given the identity of the nearest neighbour, for each individual emperor tamarin at TZ. Numbers in bold represent hourly scent-marking frequencies weighted for the proportion of time the pair spent in proximity, used to create the sociogram in **Fig. II.7**; numbers in brackets are the original hourly scent-marking frequencies.

Emperor tamarins at TZ		**Nearest neighbour**						
		Repro. ♀	Repro. ♂	Subord. ♀	Subord. ♂	Juvenile ♀	Juvenile ♂	None
Signalling individual	Repro. ♀		**0.596** (1.320)	**0.000** (0.120)	**0.211** (0.080)	**0.983** (0.280)	**0.185** (0.040)	**0.256** (0.080)
	Repro. ♂	**0.000** (0.000)		**0.152** (0.060)	**0.317** (0.080)	**0.047** (0.040)	**0.048** (0.020)	**0.210** (0.080)
	Subord. ♀	**0.399** (0.120)	**0.425** (0.120)		**0.040** (0.020)	**0.000** (0.160)	**0.397** (0.080)	**0.112** (0.040)
	Subord. ♂	**0.000** (0.000)	**1.064** (0.240)	**0.075** (0.040)		**0.287** (0.100)	**0.040** (0.020)	**0.390** (0.060)
	Juvenile ♀	**0.095** (0.020)	**0.071** (0.020)	**0.000** (0.000)	**0.036** (0.020)		**0.196** (0.100)	**0.000** (0.000)
	Juvenile ♂	**0.071** (0.020)	**0.041** (0.020)	**0.041** (0.020)	**0.000** (0.000)	**0.028** (0.020)		**0.000** (0.020)

Table B.3. Weight matrix representing hourly scent-marking frequency given the identity of the nearest neighbour, for each individual cotton-top tamarin at DMP. Numbers in bold represent hourly scent-marking frequencies weighted for the proportion of time the pair spent in proximity, used to create the sociogram in **Fig. II.8**; numbers in brackets are the original hourly scent-marking frequencies.

Cotton-top tamarins at DMP		**Nearest neighbour**				
		Repro. ♀	Repro. ♂	Juvenile ♂1	Juvenile ♂2	None
Signalling individual	Repro. ♀		**3.215** (1.320)	**3.210** (1.720)	**1.754** (1.754)	**1.001** (1.001)
	Repro. ♂	**0.000** (0.280)		**0.288** (0.180)	**0.205** (0.205)	**0.159** (0.159)
	Juvenile ♂1	**0.448** (0.180)	**0.169** (0.060)		**0.016** (0.016)	**0.000** (0.000)
	Juvenile ♂2	**0.063** (0.040)	**0.135** (0.040)	**0.020** (0.020)		**0.000** (0.000)

Table B.4. Weight matrix representing hourly scent-marking frequency given the identity of the nearest neighbour, for each individual cotton-top tamarin at PWP. Numbers in bold represent hourly scent-marking frequencies weighted for the proportion of time the pair spent in proximity, used to create the sociogram in **Fig. II.9**; numbers in brackets are the original hourly scent-marking frequencies.

Cotton-top tamarins at PWP		**Nearest neighbour**						
		Repro. ♀	Repro. ♂	Subord. ♀	Subord. ♂	Juvenile ♀	Juvenile ♂	None
Signalling individual	Repro. ♀		**1.052** (1.340)	**0.513** (0.100)	**1.347** (0.160)	**0.264** (0.120)	**0.289** (0.100)	**0.452** (0.060)
	Repro. ♂	**0.256** (0.020)		**0.103** (0.040)	**0.000** (0.000)	**0.318** (0.160)	**0.349** (0.120)	**0.099** (0.060)
	Subord. ♀	**0.725** (0.240)	**0.965** (0.260)		**1.218** (0.420)	**0.779** (0.260)	**0.828** (0.400)	**0.519** (0.040)
	Subord. ♂	**0.082** (0.040)	**0.123** (0.020)	**0.041** (0.020)		**0.127** (0.060)	**0.035** (0.020)	**0.000** (0.000)
	Juvenile ♀	**0.000** (0.000)	**0.000** (0.000)	**0.000** (0.000)	**0.000** (0.000)		**0.000** (0.000)	**0.000** (0.000)
	Juvenile ♂	**0.085** (0.020)	**0.000** (0.000)	**0.064** (0.020)	**0.114** (0.040)	**0.000** (0.000)		**0.000** (0.000)

Appendix C

Table C.1. Volatile compounds revealed by SPME–GC-MS analysis of the 95 samples included in the present analysis, and associated retention times (RT) in minutes. Compounds #01–#47 (in bold) were tentatively identified (see list of compounds of interest in **Table III.7**); compounds #48–#407 are unknown, listed in order of retention time.

#	RT range (min)	#	RT range (min)	#	RT range (min)
01	3.021–3.057	**33**	17.151–17.236	65	6.047
02	3.142–3.248	**34**	18.783–18.797	66	6.075–6.103
03	3.330–3.348	**35**	18.985–19.276	67	6.150–6.153
04	3.567–3.589	**36**	19.368–19.481	68	6.164–6.167
05	4.628–4.798	**37**	19.982–19.996	69	6.320
06	6.121–6.178	**38**	21.461–21.589	70	6.692
07	6.203–6.369	**39**	21.471–21.482	71	7.848
08	7.210–7.267	**40**	22.482–22.504	72	7.969–7.980
09	7.909–7.973	**41**	23.053–23.192	73	7.994
10	8.026–8.097	**42**	23.759–23.964	74	7.997–8.011
11	8.799–8.859	**43**	24.841–25.000	75	8.001
12	8.987–8.990	**44**	25.241–25.255	76	8.043–8.068
13	9.129–9.189	**45**	26.181–26.350	77	8.100–8.139
14	9.292–9.356	**46**	27.463	78	8.178–8.235
15	9.700–9.778	**47**	28.426–28.451	79	8.324
16	9.792–9.810	48	2.330	80	8.405
17	11.083–11.186	49	2.447	81	8.501–8.547
18	11.331–11.338	50	2.539–2.582	82	8.512
19	11.775–11.782	51	2.716–2.727	83	8.583–8.618
20	11.973–11.987	52	2.862–2.972	84	8.749
21	12.072–12.083	53	3.167–3.184	85	8.841
22	13.410–13.424	54	3.365–3.380	86	8.923–8.944
23	13.505–13.601	55	4.465–4.468	87	9.033
24	14.151–14.254	56	4.944	88	9.161–9.189
25	14.474–14.622	57	5.231–5.245	89	9.200–9.207
26	14.686–14.722	58	5.398	90	9.200
27	14.782–14.885	59	5.437	91	9.207
28	14.995–15.091	60	5.437	92	9.207
29	15.658–15.761	61	5.461	93	9.210–9.221
30	16.130–16.151	62	5.461	94	9.235
31	16.580–16.687	63	5.490	95	9.334–9.338
32	17.010–17.031	64	5.834	96	9.338

Table C.1. Continued (1/3).

#	RT range (min)	#	RT range (min)	#	RT range (min)
97	9.483–9.501	134	14.275	171	16.339
98	9.600–9.611	135	14.325–14.410	172	16.346
99	9.739	136	14.406	173	16.357–16.368
100	9.778–10.193	137	14.473	174	16.385
101	10.193	138	14.683	175	16.389
102	10.203–10.253	139	14.857	176	16.396
103	10.253–10.278	140	15.013	177	16.399
104	10.707–10.707	141	15.037–15.041	178	16.428
105	10.796–11.008	142	15.101–15.105	179	16.445
106	10.902–10.906	143	15.123–15.140	180	16.460–16.460
107	11.026–11.079	144	15.218–15.222	181	16.566–16.570
108	11.133–11.140	145	15.229–15.591	182	16.659
109	11.189–11.246	146	15.346–15.424	183	16.687–16.690
110	11.218	147	15.406	184	16.701–16.708
111	11.402–11.402	148	15.481	185	16.708–16.708
112	11.565–11.572	149	15.495–15.530	186	16.758–16.761
113	11.672–11.682	150	15.608	187	16.768–16.793
114	11.711–11.792	151	15.630	188	16.818–16.839
115	11.778	152	15.740–15.747	189	16.864
116	11.782–11.817	153	15.765–15.885	190	16.907–16.999
117	11.849–11.863	154	15.839	191	16.931–16.949
118	12.101–12.154	155	15.938–15.988	192	16.981
119	12.172–12.182	156	15.949–16.020	193	16.988–17.009
120	12.388–12.477	157	16.006	194	16.992
121	12.736–12.753	158	16.041–16.048	195	17.002–17.009
122	12.888–12.895	159	16.048	196	17.013–17.020
123	12.913–12.927	160	16.059–16.059	197	17.017
124	12.988–12.991	161	16.062	198	17.017
125	13.168–12.200	162	16.066–16.098	199	17.038–17.041
126	13.239–13.257	163	16.073–16.077	200	17.084–17.190
127	13.300	164	16.094–16.105	201	17.112
128	13.356–13.374	165	16.215–16.219	202	17.116
129	13.605–13.754	166	16.229–16.247	203	17.318–17.329
130	13.835–13.913	167	16.289–16.314	204	17.396–17.417
131	13.938–14.027	168	16.311–16.332	205	17.449–17.456
132	13.952–13.963	169	16.311	206	17.453–17.460
133	13.970–13.973	170	16.325–16.357	207	17.513

Table C.1. Continued (2/3).

#	RT range (min)	#	RT range (min)	#	RT range (min)
208	17.513	245	19.538	282	22.163
209	17.662	246	19.542	283	22.163–22.173
210	17.715–17.719	247	19.613	284	22.163
211	17.715–17.719	248	19.829–19.879	285	22.237–22.237
212	17.740	249	19.875–19.896	286	22.280
213	17.768	250	19.889	287	22.411
214	17.963	251	19.911–19.925	288	22.461
215	17.974–17.985	252	19.982–19.989	289	22.464–22.475
216	17.974–18.063	253	19.985–20.219	290	22.471–22.478
217	18.002–18.006	254	20.120	291	22.482–22.493
218	18.038–18.045	255	20.138–20.149	292	22.493–22.496
219	18.056–18.059	256	20.226–20.272	293	22.500–22.501
220	18.081	257	20.460–20.482	294	22.596–22.624
221	18.244–18.247	258	20.482	295	22.599
222	18.268–18.297	259	20.609	296	22.712–22.744
223	18.378–18.407	260	20.751–20.755	297	22.741–22.744
224	18.403–18.410	261	20.758–20.890	298	22.883–22.886
225	18.492–18.503	262	20.811–20.851	299	22.915
226	18.534–18.552	263	20.886–21.038	300	22.922
227	18.613–18.620	264	20.946	301	23.074–23.078
228	18.680–18.687	265	20.992	302	23.142
229	18.793	266	21.035	303	23.230–23.252
230	18.829–18.836	267	21.131	304	23.305
231	18.879–18.889	268	21.155–21.230	305	23.319–23.472
232	18.928	269	21.230	306	23.326–23.351
233	19.017	270	21.241–21.255	307	23.436–23.436
234	19.056–19.063	271	21.258–21.273	308	23.447–23.535
235	19.084	272	21.595	309	23.454
236	19.088	273	21.677–21.698	310	23.525
237	19.169	274	21.695–21.727	311	23.546–23.564
238	19.173	275	21.698–21.712	312	23.606
239	19.290–19.301	276	21.727	313	23.610–23.610
240	19.304–19.304	277	21.797–21.822	314	23.620
241	19.446	278	21.819	315	23.762–23.780
242	19.503–19.503	279	21.893–21.897	316	23.769
243	19.513	280	21.936–21.936	317	23.830–23.837
244	19.531	281	22.053–22.068	318	23.840–23.851

Table C.1. Continued (3/3).

#	RT range (min)	#	RT range (min)	#	RT range (min)
319	24.007	349	25.515–25.767	379	29.036–29.059
320	24.050–24.182	350	25.532–25.571	380	29.189
321	24.053–24.181	351	25.582	381	29.242
322	24.142	352	25.706–25.709	382	29.313
323	24.188	353	25.865	383	29.866–30.047
324	24.188–24.195	354	26.025	384	29.888–30.054
325	24.195	355	26.032–26.193	385	29.892–30.022
326	24.369–24.497	356	26.298	386	30.044
327	24.450–24.457	357	26.316	387	30.048
328	24.472	358	26.518–26.518	388	30.051–30.217
329	24.557–24.560	359	26.749	389	30.182–30.205
330	24.596–24.621	360	26.759–26.766	390	31.070–31.214
331	24.606–24.755	361	27.007–27.018	391	31.267–31.456
332	24.670–24.699	362	27.153–27.156	392	31.555–31.555
333	24.716–24.748	363	27.252	393	31.700–32.636
334	24.785–24.794	364	27.386	394	32.101–32.108
335	24.858–24.887	365	27.415–27.419	395	32.212
336	24.897–25.025	366	27.486–27.490	396	32.296–32.299
337	24.908	367	27.579–27.582	397	32.392–32.399
338	24.911	368	27.582–27.596	398	32.839–32.846
339	25.021–25.036	369	27.696	399	33.016–33.193
340	25.050–25.064	370	27.713	400	33.520
341	25.160	371	27.983–27.983	401	33.633–33.640
342	25.181–25.224	372	27.983–27.994	402	34.343–34.350
343	25.216–25.220	373	28.167	403	34.796
344	25.225–25.227	374	28.178–28.193	404	34.910–34.910
345	25.245	375	28.302	405	35.776–36.052
346	25.319–25.390	376	28.331	406	36.755
347	25.330–25.343	377	28.380	407	36.886
348	25.461	378	28.388		

Appendix D

Table D.1. Samples collected in 2017 from the scent-glands (i.e. anogenital, AG, suprapubic, SP, and sternal, ST) and skin of 13 groups of wild emperor tamarins, *S. imperator* (SI) and saddleback tamarins, *L. weddelli* (LW), analysed *in situ*. Compounds of interest are listed A–K (see **Table IV.3**). NA= no compound of interest found in the sample.

#	Species	Group	Sex	Repro. status	Indiv.	Sample type	Compounds of interest
1	LW	LW1	F	1ary	LW1_F1	AG	NA
2	LW	LW1	F	1ary	LW1_F1	SP	NA
3	LW	LW1	F	1ary	LW1_F1	ST	NA
4	LW	LW1	F	1ary	LW1_F1	Skin	A
5	LW	LW1	M	2ary	LW1_M1	AG	NA
6	LW	LW1	M	2ary	LW1_M1	SP	NA
7	LW	LW1	M	2ary	LW1_M1	ST	NA
8	LW	LW1	M	2ary	LW1_M1	Skin	NA
9	LW	LW1	M	1ary	LW1_M2	AG	NA
10	LW	LW1	M	1ary	LW1_M2	SP	NA
11	LW	LW1	M	1ary	LW1_M2	ST	NA
12	LW	LW1	M	1ary	LW1_M2	Skin	NA
13	LW	LW1	M	None	LW1_M3	AG	NA
14	LW	LW1	M	None	LW1_M3	SP	NA
15	LW	LW1	M	None	LW1_M3	ST	NA
16	LW	LW1	M	None	LW1_M3	Skin	NA
17	LW	LW2	F	1ary	LW2_F1	AG	NA
18	LW	LW2	F	1ary	LW2_F1	SP	NA
19	LW	LW2	F	1ary	LW2_F1	ST	NA
20	LW	LW2	F	1ary	LW2_F1	Skin	NA
21	LW	LW2	F	None	LW2_F2	AG	NA
22	LW	LW2	F	None	LW2_F2	SP	NA
23	LW	LW2	F	None	LW2_F2	ST	NA
24	LW	LW2	F	None	LW2_F2	Skin	NA
25	LW	LW2	M	2ary	LW2_M1	AG	A
26	LW	LW2	M	2ary	LW2_M1	SP	A
27	LW	LW2	M	2ary	LW2_M1	ST	F
28	LW	LW2	M	2ary	LW2_M1	Skin	NA
29	LW	LW2	M	1ary	LW2_M2	AG	NA

Table D.1. Continued (1/5).

#	Species	Group	Sex	Repro. status	Indiv.	Sample type	Compounds of interest
30	LW	LW2	M	1ary	LW2_M2	SP	NA
31	LW	LW2	M	1ary	LW2_M2	ST	A
32	LW	LW2	M	1ary	LW2_M2	Skin	NA
33	LW	LW2	M	None	LW2_M3	AG	NA
34	LW	LW2	M	None	LW2_M3	SP	NA
35	LW	LW2	M	None	LW2_M3	ST	NA
36	LW	LW2	M	None	LW2_M3	Skin	NA
37	LW	LW3	F	1ary	LW3_F2	SP	NA
38	LW	LW3	F	1ary	LW3_F2	ST	NA
39	LW	LW3	F	1ary	LW3_F2	Skin	NA
40	LW	LW3	M	2ary	LW3_M1	AG	NA
41	LW	LW3	M	2ary	LW3_M1	SP	A
42	LW	LW3	M	2ary	LW3_M1	Skin	NA
43	LW	LW3	M	2ary	LW3_M2	AG	NA
44	LW	LW3	M	2ary	LW3_M2	SP	NA
45	LW	LW3	M	2ary	LW3_M2	ST	A
46	LW	LW3	M	2ary	LW3_M2	Skin	NA
47	LW	LW4	F	1ary	LW4_F2	SP	NA
48	LW	LW4	F	1ary	LW4_F2	ST	NA
49	LW	LW4	F	1ary	LW4_F2	AG	NA
50	LW	LW4	F	1ary	LW4_F2	Skin	NA
51	LW	LW4	M	None	LW4_M1	AG	NA
52	LW	LW4	M	None	LW4_M1	SP	NA
53	LW	LW4	M	None	LW4_M1	ST	NA
54	LW	LW4	M	None	LW4_M1	Skin	NA
55	LW	LW4	M	1ary	LW4_M2	AG	NA
56	LW	LW4	M	1ary	LW4_M2	SP	NA
57	LW	LW4	M	1ary	LW4_M2	ST	NA
58	LW	LW4	M	1ary	LW4_M2	Skin	NA
59	LW	LW4	M	1ary	LW4_M3	AG	NA
60	LW	LW4	M	1ary	LW4_M3	SP	NA
61	LW	LW4	M	1ary	LW4_M3	ST	A
62	LW	LW4	M	1ary	LW4_M3	Skin	NA
63	LW	LW5	F	1ary	LW5_F1	AG	NA
64	LW	LW5	F	1ary	LW5_F1	SP	NA
65	LW	LW5	F	1ary	LW5_F1	ST	NA
66	LW	LW5	F	1ary	LW5_F1	Skin	NA

Table D.1. Continued (2/5).

#	Species	Group	Sex	Repro. status	Indiv.	Sample type	Compounds of interest
67	LW	LW5	F	1ary	LW5_F2	AG	NA
68	LW	LW5	F	1ary	LW5_F2	SP	NA
69	LW	LW5	F	1ary	LW5_F2	ST	NA
70	LW	LW5	F	1ary	LW5_F2	Skin	NA
71	LW	LW5	F	None	LW5_F3	AG	NA
72	LW	LW5	F	None	LW5_F3	SP	NA
73	LW	LW5	F	None	LW5_F3	Skin	NA
74	LW	LW5	M	1ary	LW5_M1	AG	E
75	LW	LW5	M	1ary	LW5_M1	SP	NA
76	LW	LW5	M	1ary	LW5_M1	ST	NA
77	LW	LW5	M	1ary	LW5_M1	Skin	NA
78	LW	LW5	M	2ary	LW5_M2	AG	NA
79	LW	LW5	M	2ary	LW5_M2	SP	NA
80	LW	LW5	M	2ary	LW5_M2	ST	A
81	LW	LW5	M	2ary	LW5_M2	Skin	NA
82	LW	LW5	M	1ary	LW5_M3	AG	NA
83	LW	LW5	M	1ary	LW5_M3	SP	NA
84	LW	LW5	M	1ary	LW5_M3	ST	NA
85	LW	LW5	M	1ary	LW5_M3	Skin	NA
86	LW	LW6	F	1ary	LW6_F1	AG	B
87	LW	LW6	F	1ary	LW6_F1	SP	B
88	LW	LW6	F	1ary	LW6_F1	ST	B
89	LW	LW6	F	1ary	LW6_F1	Skin	NA
90	LW	LW6	M	2ary	LW6_M1	AG	NA
91	LW	LW6	M	2ary	LW6_M1	SP	NA
92	LW	LW6	M	2ary	LW6_M1	ST	A
93	LW	LW6	M	2ary	LW6_M1	Skin	NA
94	LW	LW6	M	2ary	LW6_M2	AG	NA
95	LW	LW6	M	2ary	LW6_M2	SP	NA
96	LW	LW6	M	2ary	LW6_M2	ST	A
97	LW	LW6	M	2ary	LW6_M2	Skin	NA
98	LW	LW6	M	None	LW6_M3	AG	NA
99	LW	LW6	M	None	LW6_M3	SP	NA
100	LW	LW6	M	None	LW6_M3	Skin	NA
101	LW	LW6	M	1ary	LW6_M4	AG	C
102	LW	LW6	M	1ary	LW6_M4	SP	NA
103	LW	LW6	M	1ary	LW6_M4	ST	NA

Table D.1. Continued (3/5).

#	Species	Group	Sex	Repro. status	Indiv.	Sample type	Compounds of interest
104	LW	LW6	M	1ary	LW6_M4	Skin	B
105	LW	LW7	F	1ary	LW7_F1	AG	NA
106	LW	LW7	F	1ary	LW7_F1	SP	K
107	LW	LW7	F	1ary	LW7_F1	ST	NA
108	LW	LW7	F	1ary	LW7_F1	Skin	NA
109	LW	LW7	M	1ary	LW7_M1	AG	NA
110	LW	LW7	M	1ary	LW7_M1	SP	NA
111	LW	LW7	M	1ary	LW7_M1	ST	NA
112	LW	LW7	M	1ary	LW7_M1	Skin	NA
113	LW	LW7	M	1ary	LW7_M2	AG	D
114	LW	LW7	M	1ary	LW7_M2	SP	NA
115	LW	LW7	M	1ary	LW7_M2	ST	NA
116	LW	LW7	M	1ary	LW7_M2	Skin	NA
117	LW	LW8	F	1ary	LW8_F1	AG	H
118	LW	LW8	F	1ary	LW8_F1	SP	H
119	LW	LW8	F	1ary	LW8_F1	ST	H
120	LW	LW8	F	1ary	LW8_F1	Skin	H
121	LW	LW8	M	1ary	LW8_M1	SP	NA
122	LW	LW8	M	1ary	LW8_M1	ST	NA
123	LW	LW8	M	1ary	LW8_M1	Skin	NA
124	LW	LW8	M	1ary	LW8_M2	AG	NA
125	LW	LW8	M	1ary	LW8_M2	SP	NA
126	LW	LW8	M	1ary	LW8_M2	ST	H
127	LW	LW8	M	1ary	LW8_M2	Skin	H
128	SI	SI1	F	1ary	SI1_F1	AG	NA
129	SI	SI1	F	1ary	SI1_F1	SP	J
130	SI	SI1	F	1ary	SI1_F1	ST	NA
131	SI	SI1	F	1ary	SI1_F1	Skin	NA
132	SI	SI1	F	2ary	SI1_F2	AG	NA
133	SI	SI1	F	2ary	SI1_F2	SP	NA
134	SI	SI1	F	2ary	SI1_F2	ST	NA
135	SI	SI1	F	2ary	SI1_F2	Skin	NA
136	SI	SI1	F	2ary	SI1_F3	AG	NA
137	SI	SI1	F	2ary	SI1_F3	SP	NA
138	SI	SI1	F	2ary	SI1_F3	ST	NA
139	SI	SI1	F	2ary	SI1_F3	Skin	NA
140	SI	SI1	M	2ary	SI1_M1	AG	NA

Table D.1. Continued (4/5).

#	Species	Group	Sex	Repro. status	Indiv.	Sample type	Compounds of interest
141	SI	SI1	M	2ary	SI1_M1	SP	NA
142	SI	SI1	M	2ary	SI1_M1	ST	NA
143	SI	SI1	M	2ary	SI1_M1	Skin	NA
144	SI	SI1	M	None	SI1_M2	AG	NA
145	SI	SI1	M	None	SI1_M2	ST	NA
146	SI	SI1	M	None	SI1_M2	Skin	NA
147	SI	SI1	M	2ary	SI1_M3	AG	NA
148	SI	SI1	M	2ary	SI1_M3	SP	NA
149	SI	SI1	M	2ary	SI1_M3	ST	NA
150	SI	SI1	M	2ary	SI1_M3	Skin	NA
151	SI	SI2	F	1ary	SI2_F1	AG	D, I, J
152	SI	SI2	F	1ary	SI2_F1	SP	NA
153	SI	SI2	F	1ary	SI2_F1	ST	NA
154	SI	SI2	F	1ary	SI2_F1	Skin	NA
155	SI	SI2	F	2ary	SI2_F2	AG	NA
156	SI	SI2	F	2ary	SI2_F2	SP	NA
157	SI	SI2	F	2ary	SI2_F2	ST	NA
158	SI	SI2	F	2ary	SI2_F2	Skin	NA
159	SI	SI2	M	1ary	SI2_M1	AG	NA
160	SI	SI2	M	1ary	SI2_M1	SP	NA
161	SI	SI2	M	1ary	SI2_M1	ST	NA
162	SI	SI2	M	1ary	SI2_M1	Skin	NA
163	SI	SI3	F	2ary	SI3_F1	AG	NA
164	SI	SI3	F	2ary	SI3_F1	SP	NA
165	SI	SI3	F	2ary	SI3_F1	ST	NA
166	SI	SI3	F	2ary	SI3_F1	Skin	NA
167	SI	SI3	F	None	SI3_F2	SP	NA
168	SI	SI3	F	None	SI3_F2	ST	NA
169	SI	SI3	F	None	SI3_F2	Skin	NA
170	SI	SI3	F	2ary	SI3_F3	AG	NA
171	SI	SI3	F	2ary	SI3_F3	SP	NA
172	SI	SI3	F	2ary	SI3_F3	ST	NA
173	SI	SI3	F	2ary	SI3_F3	Skin	NA
174	SI	SI3	M	1ary	SI3_M1	AG	G
175	SI	SI3	M	1ary	SI3_M1	SP	NA
176	SI	SI3	M	1ary	SI3_M1	ST	NA
177	SI	SI3	M	1ary	SI3_M1	Skin	NA

Table D.1. Continued (5/5).

#	Species	Group	Sex	Repro. status	Indiv.	Sample type	Compounds of interest
178	SI	SI3	M	2ary	SI3_M2	AG	NA
179	SI	SI3	M	2ary	SI3_M2	SP	NA
180	SI	SI3	M	2ary	SI3_M2	ST	NA
181	SI	SI3	M	2ary	SI3_M2	Skin	NA
182	SI	SI4	F	1ary	SI4_F1	AG	NA
183	SI	SI4	F	1ary	SI4_F1	SP	I
184	SI	SI4	F	1ary	SI4_F1	ST	NA
185	SI	SI4	F	1ary	SI4_F1	Skin	NA
186	SI	SI4	F	None	SI4_F2	SP	NA
187	SI	SI4	F	None	SI4_F2	ST	NA
188	SI	SI4	F	None	SI4_F2	Skin	NA
189	SI	SI4	F	None	SI4_F3	SP	NA
190	SI	SI4	F	None	SI4_F3	ST	NA
191	SI	SI4	F	None	SI4_F3	Skin	NA
192	SI	SI4	M	1ary	SI4_M1	AG	NA
193	SI	SI4	M	1ary	SI4_M1	SP	NA
194	SI	SI4	M	1ary	SI4_M1	ST	NA
195	SI	SI4	M	1ary	SI4_M1	Skin	NA
196	SI	SI4	M	2ary	SI4_M2	AG	NA
197	SI	SI4	M	2ary	SI4_M2	SP	NA
198	SI	SI4	M	2ary	SI4_M2	ST	NA
199	SI	SI4	M	2ary	SI4_M2	Skin	NA
200	SI	SI4	M	2ary	SI4_M3	AG	G
201	SI	SI4	M	2ary	SI4_M3	SP	NA
202	SI	SI4	M	2ary	SI4_M3	ST	NA
203	SI	SI4	M	2ary	SI4_M3	Skin	NA
204	SI	SI4	M	1ary	SI4_M4	AG	NA
205	SI	SI4	M	1ary	SI4_M4	SP	NA
206	SI	SI4	M	1ary	SI4_M4	ST	NA
207	SI	SI4	M	1ary	SI4_M4	Skin	NA
208	SI	SI5	F	None	SI5_F2	SP	NA
209	SI	SI5	F	None	SI5_F2	ST	NA
210	SI	SI5	F	None	SI5_F2	Skin	NA
211	SI	SI5	F	2ary	SI5_F3	AG	NA
212	SI	SI5	F	2ary	SI5_F3	SP	J
213	SI	SI5	F	2ary	SI5_F3	ST	NA
214	SI	SI5	F	None	SI5_F4	AG	NA
215	SI	SI5	F	None	SI5_F4	ST	NA

Appendix E

Table E.1. Samples collected in 2018 from the scent-glands (i.e. anogenital, AG, suprapubic, SP, and sternal, ST) and skin of two groups of wild emperor tamarins, *S. imperator* (SI), transported to Anglia Ruskin University and analysed in the laboratory. Compounds of interest are listed #1–#25 (see **Table IV.5**). NA= no compound of interest found in the sample.

#	Species	Group	Sex	Repro. status	Indiv.	Sample type	Compounds of interest
1	SI	SI6	F	2ary	SI6_F1	SP	#08, 15, 23
2	SI	SI6	F	2ary	SI6_F1	ST	#08, 15
3	SI	SI6	F	2ary	SI6_F1	Skin	#01, 08, 15, 23
4	SI	SI6	F	2ary	SI6_F2	AG	#02, 05, 06, 15, 16, 23
5	SI	SI6	F	2ary	SI6_F2	SP	#01, 04, 08, 15
6	SI	SI6	F	2ary	SI6_F2	ST	#01, 04, 08
7	SI	SI6	F	2ary	SI6_F2	Skin	#01, 08, 15, 23
8	SI	SI6	F	2ary	SI6_F3	SP	#01, 07, 08, 12, 15, 23
9	SI	SI6	F	2ary	SI6_F3	ST	#08, 15
10	SI	SI6	F	2ary	SI6_F3	Skin	#15
11	SI	SI6	F	2ary	SI6_F4	SP	NA
12	SI	SI6	F	2ary	SI6_F4	ST	#04, 08, 10, 11, 15
13	SI	SI6	F	2ary	SI6_F4	Skin	#07
14	SI	SI6	M	1ary	SI6_M1	ST	#01, 08, 15, 23
15	SI	SI6	M	1ary	SI6_M1	Skin	#01, 08, 10
16	SI	SI7	F	2ary	SI7_F1	AG	#08, 15
17	SI	SI7	F	2ary	SI7_F1	SP	#01, 04, 08, 14, 15, 20
18	SI	SI7	F	2ary	SI7_F1	ST	#01, 04, 08, 10
19	SI	SI7	F	2ary	SI7_F1	Skin	#01, 04, 08, 15, 23

Table E.1. Continued.

#	Species	Group	Sex	Repro. status	Indiv.	Sample type	Compounds of interest
20	SI	SI7	M	2ary	SI7_M1	AG	#01, 03, 06, 08, 14, 15, 20, 21, 22, 24, 25, 26, 27
21	SI	SI7	M	2ary	SI7_M1	SP	#08, 15, 27
22	SI	SI7	M	2ary	SI7_M1	ST	#01, 08, 15
23	SI	SI7	M	2ary	SI7_M1	Skin	#08, 15
24	SI	SI7	M	2ary	SI7_M2	AG	#01, 08, 14, 15, 20, 27
25	SI	SI7	M	2ary	SI7_M2	SP	#01, 04, 08, 15
26	SI	SI7	M	2ary	SI7_M2	ST	#08, 09, 11, 13, 14, 15, 17, 18, 19, 20, 24
27	SI	SI7	M	2ary	SI7_M2	Skin	#01, 04, 08, 15