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CHAPTER 3

Pheromones and Chemical Communication in Lizards

José Martín and *Pilar López*

3.1 INTRODUCTION

Pheromones have been defined, based on entomological studies, as chemicals or semiochemicals produced by one individual that effect a change in the physiology (‘primer’ pheromone) or behavior (‘releaser’ pheromone) of conspecifics (Karlson and Lüscher 1959). In insects and many other invertebrates, very often just one or a pair of chemical compounds acts as an exclusive pheromone attracting the opposite sex. In contrast, vertebrates often have multicomponent pheromones with a mixture of many different chemical compounds with distinctly different functions or intended receivers (Müller-Schwarze 2006). However, compounds may be mixed together in specific proportions to determine “odor profiles” of species or individuals (Johnston 2005; Wyatt 2010). The pattern of compounds in the scent of an individual may convey various signals such as sex, age, social status, group, individuality, seasonality, condition, health state, etc. Moreover, in insects, pheromones alone can directly control reproductive behavior, whereas in vertebrates, a combination of different sensory stimuli (visual, tactile, chemical, etc.) is often required to control reproduction. Therefore, in vertebrates, pheromones may be better defined as a group of active compounds in a secretion that supply information to conspecifics that may be relevant for reproductive decisions (for reviews see Mason 1992; Wyatt 2003, 2010; Müller-Schwarze 2006; Apps 2013).

Departamento de Ecología Evolutiva, Museo Nacional de Ciencias Naturales, CSIC, José Gutiérrez Abascal 2, 28006 Madrid, Spain.

Reproductive behavior of lizards was traditionally thought to be predominantly based on conspicuous visual signals, whereas the potential role of pheromones in reproduction was not considered in most studies. However, there was considerable evidence of the chemosensory abilities of most lizards, and of the widespread occurrence of multiple types of glands that secrete chemicals with the potential of being pheromones, especially during the reproductive season. Only recently, has it been recognized that in many species of lizards, pheromones (i.e., specific compounds or mixtures of compounds) or "chemical signals" (i.e., undetermined chemical secretions) are very important, and sometimes required, for species and sex recognition, intrasexual relationships between males, social organization, territorial marking, and mate choice of lizards (reviewed in Halpern 1992; Mason 1992; Cooper 1994; Johansson and Jones 2007; Houck 2009; Mason and Parker 2010; Martín and López 2011).

3.2 CHEMOSENSORY ABILITIES OF LIZARDS

Lizards, and most tetrapods, can use their chemosensory senses to detect and discriminate many different scents in their environments coming from prey, conspecifics and/or predators. These abilities are based on the possession of higly developed olfactory and vomeronasal organs (Halpern 1992; Mason 1992; Cooper 1994; Schwenk 1995; Halpern and Martínez-Marcos 2003). The olfactory and vomeronasal systems do not have independent functions, as it was though in the past, but show deep anatomical and functional interrelationships (Halpern and Martínez-Marcos 2003; Ubeda-Banon *et al*. 2011). In many cases, scent stimuli are first received through the nares and processed by the nasal organs, and this triggers tongue-flick mediated vomerolfaction (Halpern 1992; Cooper 1994; Schwenk 1995). The vomeronasal organ sends specific chemical signals to the central nervous system activating accessory olfactory pathways. In particular, chemical compounds with a putative pheromonal function stimulate brain areas involved in sexually dimorphic reproductive behavior.

Associated with chemoreception, tongue-flicking (TF) is a characteristic behavior of lizards and snakes in which the tongue is extruded to sample chemicals from the environment that are delivered into the mouth and transported to the vomeronasal organ (Schwenk 1995). This is an easily observable and quantifiable behavior that has been used as a bioassay for chemosensory discrimination abilities of lizards and snakes (Cooper and Burghardt 1990; Cooper 1994, 1998). Different chemical stimuli impregnated in cotton swabs, tiles or papers are randomly presented close to the snout of experimental subject, and TF rates are measured during a certain time period. Detection of a scent stimulus is inferred by an increase of TF rates in response to the presentation of a given scent above the baseline TF rates observed under the experimental conditions in response to an odorless control (e.g., deionized water). Differential TF rate to different scent stimuli is considered as an indication of discrimination of the different stimuli because these elicit different responses (Cooper and Burghardt 1990; Cooper 1994, 1998). Usually, a higher TF rate indicates a higher "interest" for a given stimulus, which, depending on the context, is often considered a proxy of preference of that scent (e.g., different prey types or potential mates), or an indication that the stimulus is novel and elicits a longer chemosensory investigation (e.g., familiar vs. unfamiliar conspecific recognition). Pungency controls, such as cologne, are often used to assess responses to odorous, readily detectable chemicals, which are not relevant to the discrimination being studied (Cooper 1998; Cooper *et al*. 2003). Differences in latencies to the first TF after presentation of the scent stimulus are also used to indicate detection and discrimination of different stimuli.

In the case of lizards that do not usually tongue-flick during swab tests, such as some iguanids, other similar quantifiable chemosensory behaviors such as labial-licking, chin-rubbing or gular pumping are used (e.g., Wilgers and Horne 2009). To assess the preference, or avoidance, of particular stimuli, such as scent-marks from different individual males, many tests measure changes in behavior (e.g., locomotory activity) or time spent by the experimental lizard in different areas, or refuges, with substrates labeled with different chemical stimuli (e.g., Aragón *et al*. 2001c; Bull *et al*. 2001; Martín and López 2006a).

3.3 CHEMICAL COMPOUNDS AS POTENTIAL LIZARD PHEROMONES

Lizards have several possible sources of chemical compounds that may potentially function as pheromones, such as the skin and secretions by large specialized holocrine glands (e.g., precloacal/preanal or cloacal/urodeal glands) (Mason 1992; Labra *et al*. 2002). Reproductive hormones, such as testosterone, regulate the secretory activity of these glands (Fergusson *et al*. 1985; Mason 1992; Moore and Lindzey 1992), which indicates their role in reproduction. Some studies use gas chromatography coupled with mass spectrometry (GC-MS) for identification and quantification of lipophilic compounds in secretions (Fig. 3.1). Less frequently, the proteinacious fractions of secretions have been studied with different electrophoresis techniques, especially in the past. Studies using both methods have described the mixtures of chemical compounds secreted by lizard glands in a few lizard species from limited taxonomic groups (reviewed in Weldon *et al*. 2008) (Table 3.1).

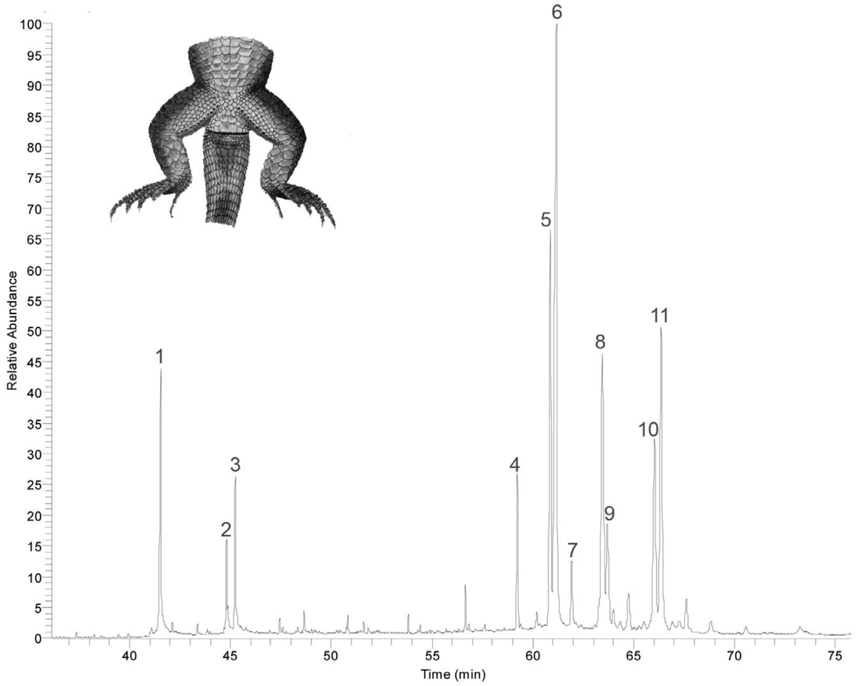

Fig. 3.1 A typical chromatogram of the lipophilic fraction of femoral gland secretions of the ocellated lizard, *Timon lepidus* (Lacertidae). The identification of the peaks of the major compounds are indicated: 1: Hexadecanoic acid; 2: Octadecenoic acid; 3: Octadecanoic acid; 4: γ-Tocopherol; 5: Cholesterol; 6: α-Tocopherol; 7: Cholestan-3-one; 8: Campesterol; 9: Ergostanol; 10: Sitosterol; 11: Ergostanol, methyl derivative. Original.

3.3.1 Skin Lipids

The skin of lizards typically contains mainly fatty acids, hydrocarbons, alcohols, steroids and waxy esters, among others. The characteristic combinations of chemicals and their concentrations vary among species (Roberts and Lillywhite 1980; Weldon and Bagnall 1987; Mason and Gutzke 1990). The main role of these lipids is to protect the skin against water loss (Roberts and Lillywhite 1980). Nevertheless, skin compounds might also be used as pheromones, at least for species, and sex recognition in short-distance interactions in many species. For example, female leopard geckos, *Eublepharis macularius* (Eublepharidae), elicit courtship behavior from males, but when females are shedding the skin, males respond aggressively, as if females were competitor males. These responses may be explained by the presence in the skin of females, but not in the skin of males, of long-

Table 3.1 Lizard and tuatara species for which lipophilic chemical compounds, with the potential of being pheromones, found in gland secretions (mostly femoral or preclocal/preanal glands except those indicated) have been described.

INFRAORDER, Family	Species	Author
IGUANIA:		
Agamidae	*Acanthocercus atricollis*	Martín *et al*. 2013c
	Uromastyx aegyptia	Martín *et al*. 2012
	Uromastyx hardwickii	Chauham 1986
Crotaphytidae	*Crotaphytus bicinctores*	Martín *et al*. 2013b
Iguanidae	*Iguana iguana*	Weldon *et al*. 1990; Alberts *et al*. 1992a,b
	Dipsosaurus dorsalis	Alberts 1990
Liolaemidae	*Liolaemus* spp. (20 species)	Escobar *et al*. 2001
	Liolaemus fabiani	Escobar *et al*. 2003
GEKKOTA:		
Gekkonidae	*Cyrtopodion scabrum*	Khannoon 2012
	Hemidactylus flaviviridis	Chauham 1986; Khannoon 2012
	Hemidactylus turcicus	Khannoon 2012
SCINCOMORPHA:		
Cordylidae	*Cordylus giganteus* (femoral and generational glands)	Louw *et al*. 2007, 2011
Teiidae	*Tupinambis merianae*	Martín *et al*. 2011
Lacertidae	*Acanthodactylus boskianus*	Khannoon *et al*. 2011a, 2013
	Acanthodactylus erythrurus	López and Martín 2005d
	Iberolacerta cyreni (=Lacerta monticola cyreni)	López and Martín 2005c; López *et al*. 2006
	Iberolacerta monticola (=Lacerta monticola monticola)	Martín *et al*. 2007c; López *et al*. 2009a
	Lacerta schreiberi	López and Martín 2006
	Lacerta viridis	Kopena *et al*. 2009
	Podarcis gaigeae	Runemark *et al*. 2011
	Podarcis hispanica (species complex)	Martín and López 2006c; Gabirot *et al*. 2010a, 2012a,b
	Podarcis lilfordi	Martín *et al*. 2013a
	Podarcis muralis	Martín and López 2006c; Martín *et al*. 2008
	Psammodromus algirus	Martín and López 2006d
	Psammodromus hispanicus	López and Martín 2009a
	Timon lepidus (=Lacerta lepida)	Martín and López 2010a
	Zootoca vivipara (=Lacerta vivipara)	Gabirot *et al*. 2008

Table 3.1 contd....

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INFRAORDER, Family	Species	Author
Scincidae	*Plestiodon laticeps* (=Eumeces laticeps) (urodeal gland)	Cooper and Garstka 1987
	Egernia striolata (feces)	Bull *et al*. 1999a
Amphisbaenidae	*Blanus cinereus*	López and Martín 2005b, 2009b
RHYNCHOCEPHALIA	*Sphenodon punctatus* (cloacal gland)	Flachsbarth *et al*. 2009

chain methyl ketones, which are lost after shedding the skin (Mason and Gutzke 1990). Interestingly, similar methyl ketones are found in the skin of female garter snakes, *Thamnophis sirtalis parietalis,* where they serve as sex attractiveness pheromones (Mason *et al*. 1990).

3.3.2 Compounds in Femoral and Precloacal Gland Secretions

Many lizards have femoral or precloacal/preanal glands, which are probably homologous with each other, differing only in their position in different species (Gabe and Saint Girons 1965). These are holocrine glands that produce an abundant secretion that is slowly secreted through the epidermal pores of femoral, preanal or precloacal glands (Cole 1966; Alberts 1993). Secretion is usually more abundant in males (i.e., it is often absent in females although they have vestigial pores) and during the mating season (Alberts *et al*. 1992b; Martins *et al*. 2006), and production is stimulated by androgenic hormones (e.g., Fergusson *et al*. 1985). Owing to the ventral location of femoral and precloacal pores, secretions are passively deposited on substrates as lizards move, which may serve to scent mark territories (see Section 3.5.1). Moreover, active rubbing of the pores against substrate has been observed.

Both lipophilic and proteinaceous compounds are generally found in femoral (or precloacal) secretions. Proteins may be the major component in secretions. Although they show characteristic and stable species-dependent patterns, minor differences among them among individuals might be used in individual recognition (Alberts 1990, 1991; Alberts and Werner 1993; Alberts *et al*. 1993).

In addition to these roles of proteinaceous compounds, lipophilic compounds may be important for communication in a reproductive context (e.g., Martín and López 2006a). Lipids have the advantage of being more volatile and have a high degree of molecular diversity, which increases the potential information content of a pheromone. In addition, the production of lipids is regulated by the general metabolism, and, thus, secreted lipids could be directly related to, and thereby potentially signal, the characteristics and condition of the signaler. Typical lipophilic compounds in femoral or precloacal gland secretions of lizards are steroids and carboxylic or fatty acids, as major compounds, together with usually minor amounts of alcohols, carboxylic acid esters (=waxy esters), squalene, tocopherol, ketones, aldehydes, furanones, alkanes or amides, and other minor and less frequent compounds (reviewed in Weldon *et al*. 2008).

3.3.2.1 Steroids

Among the lipids found in gland secretions of lizards, steroids are usually the most abundant, with cholesterol being in many cases the main compound (Weldon *et al*. 2008). However, it is likely that cholesterol or other steroids are, at least initially, only useful to form an unreactive apolar "matrix" that holds and protects other lipids in the scent marks (Escobar *et al*. 2003). Nevertheless, the relative amount of cholesterol, for example, may depend on body size in male rock lizards, *Iberolacerta cyreni* (Lacertidae), suggesting a signaling function in male intrasexual relationships (Martín and López 2007; see Section 3.5.2).

Every lizard species seems to have a specific combination of steroids that appear in roughly similar relative proportions in secretions of all individuals, although there is interindividual variability in the exact proportions of each steroid. Cholesterol is the most abundant steroid in many species but not in others. For example, in green lizards, the main steroids are ergostanol and cholestanol in the Schreiber's green lizard, *Lacerta schreiberi* (López and Martín 2006) and cholestanol and cholesterol in the ocellated lizard, *Timon lepidus* (Martín and López 2010a) (Fig. 3.1). Campesterol is the main steroid in *Psammodromus* spp. (Lacertidae) lizards (Martín and López 2006d; López and Martín 2009a). In the green iguana, *Iguana iguana* (Iguanidae), lanosterol is the most abundant steroid, followed by campesterol and cholesterol (Weldon *et al*. 1990; Alberts *et al*. 1992a). In *Liolaemus* spp. (Lioalemidae) lizards, cholesterol and cholestanol are the main steroids (Escobar *et al*. 2001). In the Great Basin collared lizard, *Crotaphytus bicinctores* (Crotaphytidae), in addition to the ubiquitous cholesterol, two triunsaturated steroids, cholesta-2,4,6-triene and cholesta-4,6,8(14)-triene, are the other two main steroids in secretions (Martín *et al*. 2013b). Other steroids, such as cholesta-3,5-diene, stigmasterol, cholestan-3-one and sitosterol are also commonly found in secretions of many lizards in lower proportions (Weldon *et al*. 2008), together with a large variety of derivatives and unidentified (probably unknown) steroids. Many of these steroids are of vegetal (=phytosterols) or microbial origin that have to be obtained from the diet, suggesting a relationship between diet and characteristics of gland secretions. Interestingly, some lizards secrete steroids that are precursors of vitamins, such as cholesta-5,7-dien-3-ol (=dihydrocholesterol; a precursor of vitamin D_3) and ergosterol (provitamin D_2). Thus, diet quality may affect quality of pheromones, which may explain why, in some lacertid lizards, females prefer the scent of males with high proportions of these provitamins (López and Martín 2005a; Martín and López 2006a,b; see Sections 8.5.3 and 8.6).

3.3.2.2 Fatty acids

Fatty or carboxylic acids, both saturated and unsaturated, are abundant in most glandular secretions of lizards. Hexadecanoic (=palmitic) and octadecenoic (=oleic) acids are present in most lizard species. Other fatty acids commonly found, although in lower proportions, are tetradecanoic (=myristic), hexadecenoic (=palmitoleic), octadecanoic (=stearic), 9,12-octadecadíenoic (=linoleic), and eicosanoic (=arachidic) acids, among others (Weldon *et al*. 2008).

The fatty acids are typically found in series, which vary with respect to the number of carbons that form the hydrocarbon chain and vary among species. This has been interpreted as an adaptation to maximize efficacy of substrate scent marks under different microclimatic conditions, with fatty acids of high molecular weight, and, therefore, less volatile, being favored in areas with higher temperatures or greater humidity (Alberts 1992). Fatty acids can also appear in the form of ethyl esters, which confer more stability. For example, in *Iguana iguana*, from warm wet tropics, the chain lengths of fatty acids found in femoral secretions range between C_{14} and C_{26} (Weldon *et al*. 1990; Alberts *et al*. 1992a), while *Iberolacerta cyreni* lizards from cold, dry high mountains, have fatty acids between C_6 and C_{22} (López and Martín 2005c). Also, in the Iberian wall lizard, *Podarcis hispanica* (Lacertidae), populations from relatively dryer habitats with mild temperatures have a higher proportion of fatty acids of low molecular weight (Martín and López 2006c). However, under the warmest and driest conditions, where evaporation rates are higher, *P. hispanica* also have the more stable ethyl ester forms of fatty acids (Gabirot *et al*. 2012a). In contrast, in the Spanish sand lizard, *Psammodromus hispanicus* (Lacertidae), which inhabits grassy substrates where scent marks could be useless, there is a great abundance of fatty acids with a low number of carbons, especially dodecanoic acid, which is highly volatile and, thus, might be more suitable for short-distance communication not requiring a durable signal (López and Martín 2009a).

Within the same species and population, dietary or hormonal differences among individuals might result in different proportions of fatty acids. For example, in *Iberolacerta cyreni*, proportions of oleic acid in femoral secretions of males were positively related to body condition of males, suggesting that the amount of oleic acid secreted may reflect the amount of body fat reserves of a male (Martín and López 2010b). Also, in *Iguana iguana*, the proportion of unsaturated fatty acids increases during the mating season when androgens also increase, which may enhance volatility and detectability of secretions (Alberts *et al*. 1992a,b). Stressful situations, such as increased predation risk levels, may also alter proportion of lipids (fatty acids and steroids) in secretions, probably due to the increase in circulating levels of corticosterone and its effect on lipid metabolism (Aragón *et al*. 2008).

In secretions of the Argentine black and white tegu lizard, *Tupinambis merianae* (Teiidae), there are large (>25%) amounts of 9,12-octadecadienoic acid (= linoleic acid) (Martín *et al*. 2011). This unsaturated fatty acid has been found in secretions of other lizards but always in very small amounts (Weldon *et al*. 2008). Secretion of large amounts of linoleic acid must be costly for lizards because it is one of two essential polyunsaturated fatty acids that many animals must ingest for good health. Given the dietary origin and the important functions of linoleic acid in metabolism, its actual function in femoral secretions of *T. merianae* must be sufficiently important to divert it from metabolism and "secrete" it from the body. It is likely that only males able to get an adequate dietary supply could secrete it. Therefore, the presence of linoleic acid in secretions might signal male quality (see Section 8.6).

3.3.2.2 Alcohols

Glandular secretions of lacertid lizards usually also include some alcohols, but alcohols were absent in secretions of several iguanid species (although relatively few iguanids have been studied). Lacertid lizards usually have alcohols such as hexadecanol or octadecanol in low proportions, but this does not imply that alcohols are unimportant. For example, in rock lizards, *Iberolacerta monticola* (Lacertidae), males with femoral secretions with higher abundances of hexadecanol and octadecanol had higher dominance status, and males respond aggressively to these alcohols (Martín *et al*. 2007c; see Section 3.5.2). In spiny-footed lizards (*Acanthodactylus erythrurus* and *A. boskianus*) (Lacertidae), long-chain alcohols (e.g., hexacosanol and tetracosanol) are the most abundant compounds (hexacosanol is also known as 'ceryl alcohol', and tetracosanol as 'lignoceric alcohol'). These alcohols may form waxy esters that make femoral secretions more cohesive, enhancing durability of pheromonal signals in the dry habitat of these lizards (López and Martín 2005d; Khannoon *et al*. 2011a). These alcohols may also be involved in signaling dominance, as suggested by the avoidance or aggressive behaviors of male *A. boskianus* lizards in response to these compounds (Khannoon *et al*. 2011b).

3.3.2.3 Other lipophilic compounds

Femoral or precloacal secretions of lizards also contain other types of compounds, usually as minor components, but in some cases as major compounds. Even if they are not especially abundant, such compounds are potentially important in communication, either directly or by enhancing the signaling function of other compounds.

Esters of a long chain fatty acid and a long chain fatty alcohol (= waxy esters) are found in secretions of many lizards (Weldon *et al*. 2008). Usually, there are diverse esters of the fatty acids hexadecanoic (=hexadecanoates), octadecenoic (=octadecenoates) and octadecanoic acids (=octadecanoates), linked to alcohols such as tetradecanol, hexadecanol or octadecanol. These are waxy compounds that may confer a greater stability to secretions, allowing scent marks to persist longer in very dry and warm or very humid environments. For example, waxy esters of fatty acids are especially abundant in femoral secretions of *Crotaphytus bicinctores*, in which the high proportion of waxy esters derived from the long chain eicosanoic (=icosanoates) and docosanoic acids (=docosanoates) is noteworthy (Martín *et al*. 2013b). This abundance of more stable waxy esters may protect scent marks from rapid evaporation in the xeric warm conditions in the habitat of this lizard.

Squalene is a hydrocarbon and a triterpene, and is a natural and vital part of the synthesis of all plant and animal sterols, including cholesterol, steroid hormones, and vitamin D. It is a common constituent in secretions of many lizards (Weldon *et al*. 2008), in which it might have a role as an antioxidant. For example, in the common lizard, *Zootoca vivipara* (Lacertidae) (formerly *Lacerta vivipara*), the lipids in femoral secretions would oxidize very quickly under the humid conditions of its environments (e.g., wet meadows, swamps, damp forests, etc.), but squalene might stabilize the other lipid fractions by limiting oxidation (Gabirot *et al*. 2008). Chemosensory discrimination of sex in the fossorial amphisbaenian *Blanus cinereus,* which shows precloacal gland secretions in both sexes, may be based on the much greater proportions of squalene found in secretions of males (López and Martín 2005b). The detection of squalene that "signals" male identity elicits, only in males, aggressive responses similar to those observed in agonistic interactions between males in a reproductive context (López and Martín 2009b).

Tocopherol (=vitamin E) is the main compound in femoral secretions of green lizards (*Lacerta schreiberi, L. viridis* and *Timon lepidus*) (López and Martín 2006; Kopena *et al*. 2009; Martín and López 2010a) (Fig. 3.1), but it is also found in other lizards in much lower amounts (López and Martín 2005d; Martín and López 2006c; Gabirot *et al*. 2008). Tocopherol is a typical antioxidant that may protect other compounds in the secretions from oxidation in wet environments, but it may also have a signaling function in female mate choice (Kopena *et al*. 2011; see Section 3.6).

Ketones can also appear in minor proportions in secretions of some lizards. They might have an important role, as yet untested, in communication in some cases. For example, the presence of a series of C_{17}–C_{25} saturated methyl ketones with mostly odd-numbered carbon chains is noteworthy in preanal gland secretions of male blue-headed tree agamas, *Acanthocercus atricollis* (Agamidae) (Martín *et al*. 2013c). A similar bishomologous series of methyl ketones were found, apparently homplasically, in the femoral gland secretions of the phylogenetically distantly unrelated South African giant girdled lizard, or sungazer, *Cordylus giganteus* (Cordylidae) (Louw *et al*. 2007) and in the skins of females geckos *Eublepharis macularius* (Mason and Gutzke 1990) and female *Thamnophis sirtalis* snakes (Mason *et al*. 1990). In the latter, they have a prominent role in the social and sexual behavior.

Aldehydes, such as tetradecanal or hexadecanal, are also often found in secretions (Weldon *et al*. 2008). These are highly odoriferous compounds that might facilitate detection by conspecifics of femoral secretions after they are deposited. Aldehydes have been found in some lizard species, but not in other phylogenetically related species (e.g., they are abundant in *Psammodromus algirus* but do not appear in *P. hispanicus*; Martín and López 2006d; López and Martín 2009a). This difference suggests the hypotheses that presence of aldehydes in secretions might depend on the environment or social behavior of each species.

Other minor compounds such as amides (e.g., octadecenamide) and furanones (= lactones of fatty acids) have also been found in many lizards (Weldon *et al*. 2008). Furanone derivatives are frequently found in nature as pheromones, flavor compounds or secondary metabolites, but their potential function in lizard secretions is unknown. Also, a large number of homologous long-chain alkanes were identified in the precloacal secretions of 20 *Liolaemus* lizard species (Escobar *et al*. 2001, 2003). However, alkanes have not been found in the secretions of other lizards. In *Liolaemus* the alkanes might have come from the skin surrounding the precloacal pores rather than from the precloacal secretion *per se*. Nevertheless, pentacosane was found in femoral secretion of *Cordylus giganteus* (Louw *et al*. 2007) and octacosane, nonacosane, triacontane and hentriacontane in the Balearic lizard, *Podarcis lilfordi* (Lacertidae) (Martín *et al*. 2013a). Finally, monoglycerides of fatty acids and glycerol monoethers of long chain alcohols were identified in femoral secretions of *Acanthodactylus boskianus* (Khannoon *et al*. 2011a). These compounds have been rarely identified from nature, have not yet been found in other lizards, and their possible signaling function is unknown.

3.3.3 Compounds in Generation Glands

In addition to femoral glands, cordylid lizards have generation glands as a potential source of pheromones (Van Wyk and Mouton 1992). These glands are formed by holocrine secretory cells located in the beta-layer of the epidermis, and may occur in different body locations, such as in the femoral, precloacal, antebrachial (forearm), and dorsal epidermal regions. A chemical analysis of the secretion of generation glands of *Cordylus giganteus*, identified alkenes, carboxylic acids, alcohols, ketones, aldehydes, esters, amides, nitriles and steroids (Louw *et al*. 2011). The most abundant compound was hexadecanoic acid. Interestingly, there are important differences with compounds identified in the femoral gland secretions of this lizard species (Louw *et al*. 2007). Cholesterol, a major component in femoral secretions does not occur in the generation glands, while alkanes do not occur in femoral secretions. These differences were explaining because generation glands are glandular scales, forming part of the lizard's skin.

3.3.4 Compounds in Cloacal Secretions and Feces

Little is known about the functions of the several glands (urodeal, proctodeal, etc.) found in the cloacas of lizards. The initial function of these glands seems to be provision of lubrication to the intestinal tract to facilitate expulsion of excrements or to facilitate mating. However, the glands also are a potential source of pheromones (Trauth *et al*. 1987; Cooper and Trauth 1992). This is a relatively little explored topic, but cloacal secretions might be of great importance in chemosensory communication, especially in the groups of lizards lacking femoral or precloacal glands (e.g., Gonzalo *et al*. 2004), or in species in which females have vestigial pores with little secretion. For example, in the broad-headed skink, *Plestiodon laticeps* (Scincidae), the dorsal cloacal glands may produce a species-identifying pheromone present in both sexes that may be useful to discriminate among conspecific male sexual competitors in *P. laticeps* and closely related skinks (Cooper *et al*. 1986; Cooper and Vitt 1987; Trauth *et al*. 1987). The urodaeal gland of female black-lined plated lizards, *Gerrhosaurus nigrolineatus* (Gerrhosauridae) was hypothesized to be a source of female sex pheromone, while the dorsal and ventral glands may be the source of species-identification or male pheromones (Cooper and Trauth 1992). The precise chemical identity of such pheromones is unknown. However, when different chemical fractions of the whole urodaeal glands of female *P. laticeps* were presented to males, their tongue-flick rates were higher in response to neutral lipids than to other fractions (Cooper and Garstka 1987). Pheromonal activity appears to reside in the neutral lipid fraction, which includes steryl and wax esters and mono-, di- and triacylglycerols, but not in acidic or basic lipids, or in carbohydrate or the protein fractions.

The cloacal gland secretion of the tuatara, *Sphenodon punctatus*, contains a glycoprotein and a complex mixture of triacylglycerols derived from unusual medium chain-length fatty acids as major constituents (Flachsbarth *et al*. 2009). However, it is not clear that these compounds can function as pheromones, because tongue-flicking is not observed in social interactions of tuataras (Gans *et al*. 1984), although it remains possible that olfaction might be used because tuataras respond by biting to swabs impregnated with prey chemicals (Cooper *et al*. 2001).

In several lizard species, chemicals with pheromonal function, probably coming from the cloacal glands, may be secreted onto the surface of the feces or scats as these are deposited by the lizard. Compounds in feces seem to be useful for scent-marking and conspecific recognition. Scent from feces may provide information on familiarity, relatedness, or body size of the producer (Duvall *et al*. 1987; Carpenter and Duvall 1995; López *et al*. 1998; Bull *et al*. 1999a,b, 2001; Aragón *et al*. 2000; Moreira *et al*. 2008; Wilgers and Horne 2009). Compounds from feces with properties of pheromones have not been identified, but they are probably a combination of several lipids as, in the tree skink, *Egernia striolata* (Scincidae), they are contained in scat extracts made with organic solvents (dichloromethane); fractionation of the scats with different solvents (pentane and methanol) led to loss of the unique signals needed for individual recognition (Bull *et al*. 1999a).

Finally, compounds from cloacal glandular secretions that have pheromonal activity may be added to copulatory plugs of males. Male *Iberolacerta monticola*, can distinguish their own copulatory plugs from those of other males and can even assess the dominance status of other males by chemosensory cues from copulatory plugs (Moreira *et al*. 2006). This suggests that copulatory plugs may allow males to "scent-mark" the female body during copulations and that this behavior may influence mating decisions of other males under selective pressures of sperm competition (e.g., a male might recognize and avoid displacing its own plugs while displacing plugs of other males). These hypotheses remain to be tested.

3.4 CHEMOSENSORY RESPONSES TO SPECIFIC CHEMICAL COMPOUNDS

Many studies have shown the ability of lizards to use their chemosensory systems to discriminate the scent of conspecifics from scents of heterospecifics (e.g., Cooper and Vitt 1987; Barbosa *et al*. 2006; Gabirot *et al*. 2010a,b), scents of males from females (e.g., Cooper and Trauth 1992; Cooper and Steele 1997; Cooper *et al*. 1996; Labra and Niemeyer 1999; López and Martín 2001a; Khannoon *et al*. 2010) and reproductive condition of females (e.g., Cooper and Vitt 1984; Cooper and Pérez-Mellado 2002). Lizards also use pheromones to discriminate the scent of familiar from unfamiliar individuals and self-recognition (e.g., Alberts and Werner 1993; Cooper *et al*. 1999; Aragón *et al*. 2001a,b; Carazo *et al*. 2008).

Only a few studies have examined whether lizards can discriminate between the different types of chemical compounds found in these scents. Some studies used the TF rates of lizards to scent stimuli presented on cotton swabs to examine, within a foraging context, discrimination of compounds found in the insect prey of lizards (Cooper and Pérez-Mellado 2001; Cooper *et al*. 2002a,b). *Podarcis lilfordi*, can discriminate between lipids, proteins, and carbohydrates (Cooper *et al*. 2002a) and also among different lipids, such as glycerol, cholesterol, and oleic and hexadecanoic acids (Cooper *et al*. 2002b).

With respect to compounds found in glandular secretions of lizards, another study measured the TF responses of female *Podarcis hispanica* to two lipids (cholesterol and cholesta-5,7-dien-3-ol) (Martín and López 2006e). These steroids are major compounds in femoral secretion of males (Martín and López 2006c). Females discriminate between these two lipids, showing higher TF responses to cholesta-5,7-dien-3-ol and are able to assess differences in its concentration, responding more strongly to higher concentrations. These results, together with the female preference for areas scent marked by males with higher proportions of this steroid in their secretions (López and Martín 2005a; see Section 3.5.3), suggest that cholesta-5,7-dien-3-ol is a "key" pheromonal compound for this lizard.

In some cases, intersexual differences in chemosensory responses suggest that different compounds may carry different messages for males and females. Thus, female *Iberolacerta cyreni* have higher TF responses to cholesta-5,7-dien-3-ol and to ergosterol than to cholesterol, whereas the opposite is found in males (Martín and López 2006a, 2008a). This is probably explained by the preference of females for scent marks of males with higher proportions of cholesta-5,7-dien-3-ol and ergosterol in their secretions, which is related to the "quality" of those males (Martín and López 2006a,b; see Section 3.5.3), whereas in males cholesterol might signal the body size of a potential male opponent (Martín and López 2007; see Section 3.5.2).

Female *Iberolacerta cyreni* can also discriminate among fatty acids found in femoral gland secretions of males, such as oleic acid and hexadecanoic acid, and can assess differences in their concentration (Martín and López 2010b). This discrimination might be important for females because the amount of fatty acids secreted may reflect the amount of body fat reserves of a male. The presence of both saturated (e.g., hexadecanoic) and unsaturated (oleic) fatty acids in the males' secretions might allow the scent signal to function in varying environmental conditions because at ambient temperatures, unsaturated fatty acids may be accessible as liquids, whereas saturated fatty acids may be waxes. Thus, it is likely that females actually responded to the whole mix of fatty acids usually found in males' secretion or that under different temperature conditions, some fatty acids were more effective than others in eliciting chemosensory exploration of females.

Alcohols can also be detected by lizards. Male and female *Podarcis hispanica* lizards can discriminate among alcohols found in secretions of males and vary tongue-flick rates with their concentrations (Gabirot *et al*. 2012c). Male *Iberolacerta monticola* discriminate between different concentrations of hexadecanol, a major compound in glandular secretions of males, from other chemicals (Martín *et al*. 2007c). Moreover, males respond aggressively to hexadecanol, but respond neutrally to other compounds (Martín *et al*. 2007c). These results, together with the relationship observed between femoral secretions with higher proportions of hexadecanol and dominance, suggest that hexadecanol may be a reliable status badge in this lizard. Similarly, male *Acanthodactylus boskianus* show avoidance behavior for substrates marked with cholesterol and long chain alcohol blends (both found in males' secretions), and agonistic behavior towards these stimuli, whereas females do not respond to these chemicals (Khannoon *et al*. 2011b).

The observed chemosensory responses to glandular secretions may be a consequence of response to the combined multiple effects of different compounds. For example, female *Iberolacerta cyreni* discriminate between different concentrations of ergosterol and oleic acid presented alone and exhibit the highest chemosensory exploration to high concentrations of ergosterol, whereas high concentrations of oleic acid elicit tongue-flick (TF) rates of a magnitude similar to those to low concentrations of ergosterol (López and Martín 2012) (Fig. 3.2). Moreover, the highest TF rates are directed to a mixture containing high concentrations of both compounds combined, and there is an upper-shift of the top of the dose-response curve by the combination of the two compounds, suggesting that there are additive or synergic effects of these two compounds (Fig. 3.2).

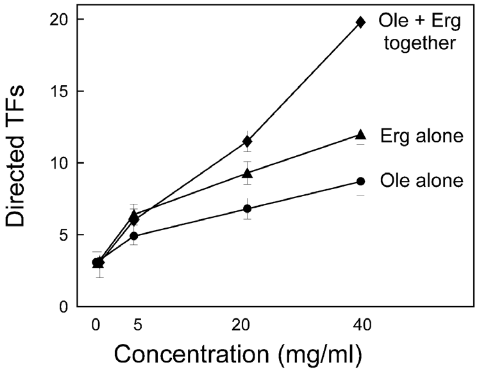

Fig. 3.2 Dose-dependent and additive effects of two compounds from males' femoral gland secretions on chemosensory responses of female Iberian rock lizards (*Iberolacerta cyreni*). Number (mean + SE) of tongue-flicks directed to swabs by female lizards in response to cotton-tipped applicators bearing different concentrations (0, 5, 20, or 40 mg/mL) of oleic acid (Ole) and ergosterol (Erg) (standard compounds) presented alone or together, all dissolved in DCM. From López, P. and Martín, J. 2012. Chemical Senses 37: 47–54. Figure 3.

3.5 REPRODUCTIVE CHEMICAL ECOLOGY IN LIZARDS

Multiple lines of evidences show that lizards have highly developed chemosensory abilities, including strong responses to scent of conspecifics, and that most lizards produce, especially during the mating season, glandular secretions that contain many chemicals that potentially function as signals. Nevertheless, as noted in Section 3.1, the reproductive behavior of lizards was long considered to be mainly based on more conspicuous visual signals. Thus, most research on reproductive behavior of lizards focused on colorful traits or movement displays. Relatively few studies have considered the potential role of chemical signals in reproductive ecology of lizards. These will be summarized in the following sections. We are just starting to understand not only the function of specific chemicals in modulating different behaviors related to reproduction and sexual selection, but also the mechanisms that explain the use and evolutionary persistence of these signals.

3.5.1 Scent-Marking

Glandular secretions, feces, or urine are very often used for scent-marking of substrates by many terrestrial vertebrates, including many lizards. These scent-marks identify territorial boundaries or attract mates (reviewed in Müller-Schwarze 2006; Mason and Parker 2010). Scent-marking a territory can be a simple and effective method to inform conspecifics about the identity and characteristics of the male that defends the marked territory. Many lizards can scent-mark their territories by using femoral or cloacal secretions and/or feces. Semiochemicals in these scent marks are known to convey information on sex, age, body size, dominance status or health condition of the signaler (reviewed in Mason 1992; Mason and Parker 2010; Martín and López 2011).

If the information in the scent-mark is reliable (e.g., Martín and López 2006b; Kopena *et al*. 2011), the signaler will benefit from this advertisement, for example, by repelling rivals or attracting mates (Martín and López 2012). Receivers of the signal may gain benefits by using information about territorial status and dominance obtained from scent-marks into their decisions about aggressive behavior toward the scent-marking male (e.g., Carazo *et al*. 2007, 2008; López and Martín 2011) or about mate choice (e.g., Martín and López 2000, 2006a,b; López *et al*. 2002b; López and Martín 2005a; Olsson *et al*. 2003). Scent-marks may be important in reproductive behavior and sexual selection of many lizards (see below).

3.5.2 Intrasexual Relationships Between Males and Social Organization

When competing for access to mates, males use cues from their rivals to judge relative fighting ability and to evaluate their chances of success in a potential future agonistic contest. In lizards, chemical signals may be a vital component of male-male contests informing males of a rival's quality or intentions. Pheromones may signal a male's dominance status, or characteristics related to fighting ability or dominance such as body size, through rates of production and/or the quality of the glandular secretions (Alberts *et al*. 1992b; López *et al*. 2003b; Martins *et al*. 2006; Martín *et al*. 2007c). In many cases, pheromones also allow lizards to discriminate between familiar and unfamiliar males and may allow true individual recognition (i.e., based on individual identity cues) (Aragón *et al*. 2001a; Carazo *et al*. 2008).

Pheromones affect intrasexual relationships in male lizards in two ways. First, pheromones deposited in substrate scent-marks can provide information in absence of the signaler on the presence of previously known individual rivals or on the fighting potential of unfamiliar individuals (Aragón *et al*. 2000, 2001a; Labra 2006; Carazo *et al*. 2007, 2008). This information may affect behavior and space-use by other males that sample the scent-marks (Alberts *et al*. 1994; Aragón *et al*. 2001c, 2003; Labra 2006). Second, pheromones may be used during actual agonistic encounters, for example, to recognize rival males (Cooper and Vitt 1987; López *et al*.

2002a). This is important because when two males interact, they become familiar and establish their relative dominance relationship, which allows them to decrease the aggressiveness in successive encounters (López and Martín 2001b). In some cases, recognition of familiar lizards or of specific individuals may be predominantly based on pheromonal cues. In *Podarcis hispanica*, resident males respond more aggressively towards unknown or familiar males experimentally impregnated with scents from unfamiliar males than to familiar males or unknown males impregnated with scents of familiar males (López and Martín 2002) (Fig. 3.3).

Chemical rival recognition may be used in other situations. Males of many species of lizards show conspicuous breeding colors, but, in some species, young or competitively inferior males conceal their sexual identity by mimicking a female-like dull coloration that allows them to evade aggression from dominant males and to adopt an alternative satellite-sneaking mating tactic. In two experiments, scent and coloration of satellite males, were manipulated in *Psammodromus algirus* (López *et al*. 2003b) and Augrabies flat lizards, *Platysaurus broadley* (Cordylidae) (Whiting *et al*. 2009). In both species, deceptive coloration was effective in avoiding aggression at long distance. However, at close range, dominant males used chemical signals to identify satellite males, as shown by aggressive responses toward satellites.

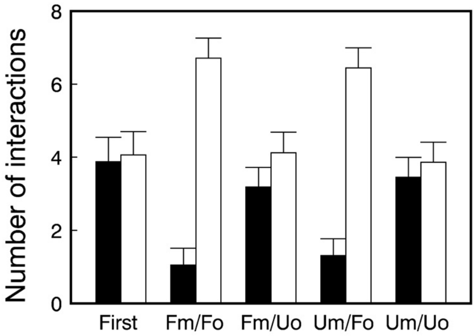

Fig. 3.3 Role of pheromones in intrasexual agonistic behavior of Iberian wall lizards (*Podarcis hispanica*). Number (mean + SE) of aggressive (black bars) and neutral (white bars) interactions in the first contest of a resident male with an intruder male impregnated with his own odor, in posterior contests with the same familiar male bearing his own odor (Fm/Fo) or impregnated with odor of an unfamiliar male (Fm/Uo), and in posterior contests with unfamiliar males impregnated with odor of a familiar male (Um/Fo) or bearing their own odor (Um/Uo). From López, P. and Martín, J. 2002. Behavioral Ecology and Sociobiology 51: 461–465. Figure 1.

In some animals, males may identify territory owners by directly comparing the scent of substrate marks with the scent of any conspecific they encounter nearby, i.e., by scent-matching (Gosling and McKay 1990). This may also occur in lizards. Thus, when an intruding male *Iberolacerta cyreni,* explores substrate scent marks, if he subsequently finds a rival male whose scent experimentally matches that of scent marks (considered presumably to be the territory owner), the intruding male delays time until the first agonistic interaction, reduces the intensity and number of fights, and wins fewer interactions than when encountering other non-matching individual males (López and Martín 2011). Therefore, males may use scent-matching as a mechanism to assess the ownership status of other males, which could contribute to modulation of further intrasexual aggressions.

However, the chemical basis of the assessment of rival dominance status or fighting ability is poorly known. Assessment might be affected by changes in concentrations of some chemicals in scents that are correlated with traits that affect fighting ability. In *Iguana iguana*, femoral glandular productivity, pore size, and the percentage of lipids in the secretions are correlated with plasma testosterone levels in dominant, although not in subordinate, adult males (Alberts *et al*. 1992b). Proportions of cholesterol in femoral secretions of male *Iberolacerta cyreni* increase with body size (López *et al*. 2006). These males discriminate chemically and respond aggressively to cholesterol stimuli presented on swabs (Martín and López 2008a), and, moreover, when cholesterol in the body scent of males is experimentally increased, they win more frequently agonistic interactions (Martín and López 2007), suggesting that high concentration of cholesterol may signal greater fighting ability linked to larger body size. This may be a reliable signal, if higher proportions of cholesterol in secretions indicate higher sex steroid (i.e., testosterone) levels that also determine aggressiveness levels (Alberts *et al*. 1992b; Sheridan 1994).

Similarly, in male *Iberolacerta monticola*, dominant males produce femoral secretions with higher proportions of two alcohols (hexadecanol and octadecanol) (Martín *et al*. 2007c) (Fig. 3.4). Males discriminate different concentrations of hexadecanol from other chemicals found in secretions and respond aggressively towards hexadecanol according to their own dominance status, but respond neutrally to other chemicals. The signal may be reliable because, given that hexadecanol elicits aggressive responses of other males, only truly dominant males with a high fighting potential should chemically signal their status. Also, it might be physiologically costly to produce femoral secretions with high amounts of hexadecanol. Consistent with this view, dominant males are healthier (i.e., have a stronger immune response), which might allow them to afford secreting greater quantities of compounds that signal a high dominance status (Martín *et al*. 2007c).

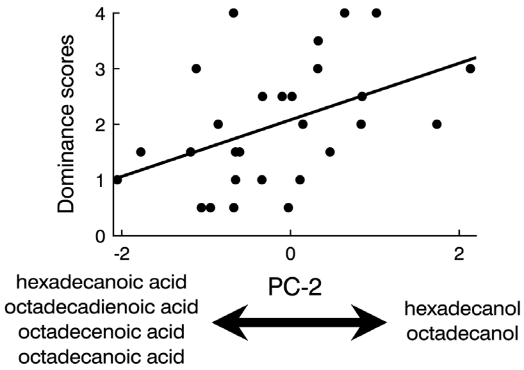

Fig. 3.4 Chemical basis of dominance status signallng in rock lizards (*Iberolacerta monticola*). Relationships between dominance status scores of male lizards and PC scores obtained from a principal components analysis on the relative proportions of chemical compoundss in femoral gland secretions. From Martín, J., Moreira, P. L. and López, P. 2007. Functional Ecology 21: 568–576. Figure 1.

3.5.3 Female Mate Choice

Some field studies suggest that females of some lizard species do not choose males, but base their space-use on the quality of a territory (e.g., thermal characteristics, abundance of food or refuges, etc.) rather than on the quality of the male that defends that territory (e.g., Hews 1993; Calsbeek and Sinervo 2002). Males would only defend these favorable territories from other males to increase their access to females. However, it is still possible that females might be attracted to a territory by male signals that may be used as "public information" to assess the quality of a territory, or through being "lured" by male signals that resemble food. In this context, in lizard species in which males scent-mark territories, pheromones may have an important role in female space-use and mate choice.

Other studies suggest that female lizards of some species might use some chemical compounds in the scent-marks of males to select areas scent marked, and, therefore, occupied by preferred potential mates (Martín and López 2006a, 2012, 2013a; Johansson and Jones 2007). On the other hand, a pre-existing sensory bias for food chemicals might also explain the chemosensory preferences of female lizards for some compounds in the scent-marks of males (Martín and López 2008). However, irrespective of the causes underlying decisions by females to spend more time in a given scent marked area, this decision about use of space will increase the probability of mating with the male that has scent marked the selected area (Martín and López 2012). Females may try to reject mating advances from "undesired" males, but males may obtain many forced matings. Therefore, space-use decisions of female lizards will have direct consequences for their reproductive success, and those space-use strategies that increase the reproductive success of females will be evolutionary selected. As a consequence, space-use decisions of female lizards based on scent marks of males may have the same evolutionary consequences as "direct" mate choice decisions of other animals.

Therefore, male lizards might use scent marks to attract females to their territories, thus increasing the probabilities of mating with these females, whereas females might use scent marks of males to select potential mates or territories of high quality. But this attracting function of the scent-marks of lizards remains little explored. One field study in *Iberolacerta cyreni*, showed that experimentally increasing ergosterol (a compound from femoral secretions of males) on rock substrates inside home ranges of males results, after some days, in increased relative densities of females, but not of males, in those areas. This effectively results in an increase of mating opportunities for resident males (Martín and López 2012) (Fig. 3.5). Also, female *I. cyreni* prefer areas scent-marked by large/old territorial males to those scent-marked by smaller/young satellite-sneaker males (López *et al*. 2003a; Martín and López 2013a) and prefer areas scent-marked by two territorial males to areas of similar size marked by a single territorial male (Martín and López 2013a). The former choice might increase the probability of obtaining multiple copulations with different males, thus favoring sperm competition and cryptic female choice, or may be a way to avoid infertile males (Martín and López 2013a).

To establish whether female mate choice exists, experimenters must select appropriate criteria base on choices used by females to select a mate (or a scent marked territory). For example, female *Podarcis hispanica*, preferentially associate with areas scent-marked by males, but females do not choose territories marked by larger versus smaller males. Taken alone, this might suggest that mate choice by females is absent in this species (Carazo *et al*. 2011). However, other experiments with this species showed that females select scents of males with higher proportions of cholesta-5,7-dien-3-ol (among scents from males of similar size), which are those with a better T-cell-mediated immune response (i.e., with a better health) (López and Martín 2005a). Similarly, female *Iberolacerta cyreni*, select areas scent-marked by males with stronger immune responses, as signaled by high ergosterol proportions in femoral secretions of males (Martín and López 2006a), or with better body condition, as signaled by high proportions of oleic acid in secretions (Martín and López 2010b). In the same way, female *Psammodromus algirus* show higher chemosensory responses to femoral gland secretions of males with low blood parasite loads and stronger immune responses, which is apparently signaled by higher proportions of

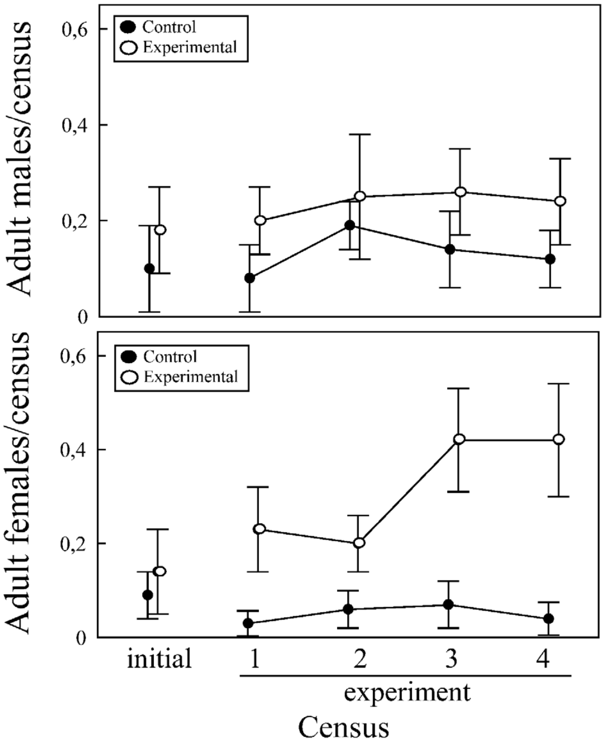

Fig. 3.5 Effects of manipulation of substrate *scent-marks* with pheromones on density of Iberian rock lizards (*Iberolacerta cyreni*). TOP: Numbers (mean + SE) of (a) adult males or (b) adult females observed in each census of the control (black circles) and experimental (open circles) plots before the experiment (initial) and during the four days after rocks were supplemented with ergosterol (experimental) or a control solution. From Martín, J. and López, P. 2012. PLoS One 7: e30108. Figure 1. BELOW: A pair of rock lizards, the territorial male (in front) has approached to a female that was probably attracted to his area by pheromones in scent-marks. Photograph by J. Martín.

two alcohols (octadecanol and eicosanol) and lower proportions of their correspondent carboxylic acids (octadecanoic and eicosanoic acids) (Martín *et al*. 2007b).

Intra- and inter-sexual competition often lead to selection for different secondary sexual traits, which may be reflected in responsiveness to male pheromones by female lizards. Scents of males that signal characteristics that confer competitive advantages to males against other males (such as a larger head or a higher bite force) are often not selected by females. Females may respond more strongly to scents of males that signal traits of a potential mate that are beneficial to females, such as a higher body condition or levels of symmetry (*Iberolacerta cyreni*, López *et al*. 2002b; Dalmatian wall lizard *Podarcis melisellensis* (Lacertidae), Huyghe *et al*. 2012).

Similarly, in sand lizards, *Lacerta agilis* (Lacertidae), females do not seem to mate selectively with larger and/or older males (Olsson and Madsen 1995), but genetic compatibility (based on the major histocompatibility complex, MHC, dissimilarity) is the main characteristic that females select based on the scent of a male (Olsson *et al*. 2003). Similar avoidance of inbreeding based on chemical signals might function in other lizard species (Bull and Cooper 1999; Bull *et al*. 2001). However, the chemical bases of this genetic discrimination remain unknown. Selective mating with non-kin or unrelated pairs may confer genetic benefits because the new combinations of immunocompetence in offspring will defend them more effectively against evolving parasites (Penn and Potts 1999). In contrast, female painted dragons, *Ctenophorus pictus* (Agamidae), do not prefer scents from unrelated males, which might be explained by weak selection against inbreeding in this species (Jansson *et al*. 2005).

3.6 EVOLUTIONARY ORIGIN OF THE CHEMICAL SIGNALING SYSTEM

Although some compounds "preferred" by females in the scent of males, or that affect intrasexual relationships between males, have been identified in a few lizard species, it is not well understood why lizards can be confident in the reliability and honesty of the message in the chemical signal (i.e., that a scent with a specific chemical characteristics always corresponds to a male with some specific characteristics). This is, however, a prerequisite for a signal to persist in evolutionary time.

One possible explanation may reside in the important metabolic organismal functions of some compounds that are, however, secreted by glands to the exterior of the body. Thus, many of the lipids commonly found in glandular secretions of lizards, such as fatty acids and steroids, function as signaling molecules or lipid mediators, show potent biological activity, and are important keys in many metabolic pathways. Some lipophilic compounds in femoral secretions of some lizards appear to be good candidates for conferring honesty to signals. These are α-tocopherol (=vitamin E), cholesta-5,7-dien-3-ol (=dihydrocholesterol; a precursor of vitamin D_3), ergosterol (=pro-vitamin D_2), 9,12-octadecadienoic acid (=linoleic acid) and 5,8,11,14-eicosatetraenoic acid (=arachidonic acid). These are essential components for metabolism that have important physiological functions. In most cases, vertebrates can only obtain them from the diet and their deficiency can cause severe disorders. However, in spite of the importance of these compounds, male lizards divert them from metabolism to allocate them for use in femoral glandular secretions. In such cases, trade-offs must exist between using these essential chemicals in metabolism and using them for scent-marking. Only males that have, or are able to obtain, an adequate supply of vitamins and essential fatty acids could allow diversion of surplus chemicals from metabolism to social signalling. Therefore, the presence of vitamins and essential fatty acids in relative high proportions in secretions might honestly advertise male quality.

For example, cholesta-5,7-dien-3-ol (=pro-vitamin D_3) is often found in the skin, where it transforms into vitamin D_3 after exposure to UV-B irradiation in sunlight. Vitamin D_3 is essential in calcium metabolism and for regulation of the immune system (Fraser 1995). However, very often, the synthesis of vitamin D_3 in the skin is not sufficient to meet physiological requirements, and lizards require dietary intake of vitamin D (Ferguson *et al*. 2005). Under these conditions, vitamin D is an essential nutrient for lizards.

Therefore, when diverting pro-vitamin D from metabolism to femoral secretions, male lizards might need to obtain more vitamin D. After supplementation of dietary vitamin D, male *Iberolacerta cyreni*, increased the proportion of pro-vitamin D_3 in femoral secretions and females preferred areas scent marked by these males with more pro-vitamin D_3 (Martín and López 2006b). This suggests that allocation of this pro-vitamin to secretions is costly and dependent on the foraging ability of a male to obtain enough vitamin D in the diet, or of the food quality within his territory, which may confer honesty to his pheromonal signal and may explain why females select the scent marked territories of these males.

Vitamin E (= α-tocopherol) is the main lipophilic antioxidant involved in membrane defence (Brigelius-Flohe and Traber 1999). This vitamin is of dietary origin and its deficiency has severe pathological consequences, such as infertility, neurological disorders and lung diseases. In the European green lizard, *Lacerta viridis*, males show high proportions of tocopherol in their femoral secretions (Kopena *et al*. 2009). These proportions increased when males were experimentally fed supplementary vitamin E, and females preferred to use areas scent-marked by these males with increased vitamin E secretion levels (Kopena *et al*. 2011) (Fig. 3.6). This suggests that the cost of

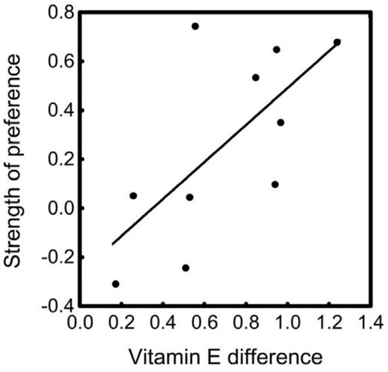

Fig. 3.6 Strength of female preference for male scent in European green lizards (*Lacerta viridis*). Female 'strength of preference' was calculated as the difference in the mean proportion of females that were observed at the areas containing chemical cues from size matched vitamin E supplemented vs. control males. Vitamin E difference is the difference in relative vitamin E content of the femoral secretions of size-matched vitamin E supplemented vs. control males within a male pair. From Kopena, R., Martín, J., López, P. and Herczeg, G. 2011. PLoS One 6: e19410. Figure 2.

allocating vitamin E to secretions, diverting it from its important organismal antioxidant function, may confer reliability to chemical signals of males. Tocopherol may be the chemical signal directly used by females; however, this antioxidant compound might simply increase duration and intensity of information provided by other signaling compounds in secretions.

Similarly, linoleic and arachidonic acids are essential fatty acids that mediate a wide range of physiological responses and maintain homeostasis, and, in spite of their important functions, are also found in secretions of some lizards in relatively high proportions (e.g., Martín *et al.* 2001). This suggests a potential, but untested, signaling function of these essential fatty acids.

Reliability of chemical signals might be also based on other mechanisms. For example, secretion of compounds could be differentially costly for different individual males, because secretion depends on testosterone levels and testosterone may have immunosuppressive effects (Folstad and Karter 1992; Belliure *et al.* 2004; Oppliger *et al.* 2004). Thus, only high quality males could afford the trade-off of producing pheromones while avoiding the detrimental effects of testosterone on their immune system. Female *Podarcis hispanica* showed higher chemosensory responses to scents of males with experimentally supplemented testosterone than to control scents (Martín *et al.* 2007a), probably because production and concentration of glandular secretions increased when testosterone increased. However, testosterone also induced a decrease in proportion of cholesta-5,7-dien-3-ol, probably as a consequence of its immunosuppressive effects (López *et al.* 2009b), and females preferred scent-marks of males that maintained high levels of this compound in secretions independently of the experimental manipulation (Martín *et al*. 2007a).

3.7 FUTURE DIRECTIONS

Although the roles of pheromones in lizard biology have been explored in the studies cited above, there is still much work to do in several fields to fully understand them. First, we need to know the compounds that may be potentially used as pheromones. Many lizard species representing diverse taxonomic lineages and geographical areas have abundant glandular secretions that have never been chemically analyzed, even for species in which glandular secretions are known to be important for chemical communication. Moreover, most available information is from femoral or precloacal glands, whereas almost nothing is known about chemicals from cloacal secretions or feces that may have pheromonal functions. Different compounds might be characteristics of some groups of lizards, but be absent in others. The presence of different compounds might have a phylogenetic effect, but it might also be linked to environmental conditions, or to the way these chemicals are used in communication (e.g., as scent-marks on different substrates, or as chemicals sampled directly from the individuals). This emphasizes the need for more studies analyzing the chemical compounds in glandular secretions of diverse groups of lizards from different habitats. These descriptive studies will allow further comparative studies to clarify the patterns of presence and abundance of compounds observed in each species, and the causes (genetic and environmental) that explain the evolution of gland secretions in lizards.

It is rarely known which specific compounds could act as pheromones. After identification of compounds in glandular secretions, further chemosensory tests should be conducted with the different compounds, alone and combined in different proportions, to understand which compounds are important in communication and how their variability in abundance affects to the chemosensory responses of conspecifics.

The next step would be to identify the "message" of these chemical compounds (i.e., the strategic element of the signal; Guilford and Dawkinds 1991). Behavioral tests are needed that relate differential responses to differences in scents of different individuals for which chemical composition of glandular secretions is known, and to differences in morphological and physiological characteristics. We need to determine which traits are sufficiently important to be signaled in each species and how they are signaled through chemical cues. Also, lizards use other sensory systems in communication, especially vision, and it may be important to understand how information contained in multiple visual and chemical signals is congruent, although sometimes used in different contexts, or different and complementary, as well as the relative importance of one type of signal or other in different species and environments.

Finally, we would need to analyze the mechanisms that allow the origin and evolutionary persistence of a chemical signal, and how the environment may drive the evolution of the chemical signaling system, especially through changes in the design of the signal to maximize its efficacy in different environmental conditions (Guilford and Dawkinds 1991). This is important, on the one hand, because if different populations of the same species use different chemical signals for reproduction, this may later lead to reproductive isolation between populations and speciation processes (e.g., Gabirot *et al*. 2012a). On the other hand, current rapid human-induced changes in the environment, such as global warming or contamination, might affect the efficacy of chemical signals with potential detrimental consequences for reproduction and stability of populations (e.g., Martín and López 2013b).

We encourage researchers to "look" for the roles of chemical signals or pheromones in the social and reproductive behavior of lizards. Not knowing these, we might be missing most of the information contained in sexual displays and would fail to understand many field observations of ecology, behavior, and evolution, of these animals.

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