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# SEX AND SEASONAL DIFFERENCES IN THE SKIN LIPIDS OF GARTER SNAKES

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**Abstract**—1. This study investigates the skin lipids of male and female red-sided garter snakes both in the breeding season and in the non-breeding season.
2. Skin lipids were analyzed by means of thin-layer chromatography (TLC) and gas chromatography/mass spectrometry (GC/MS).
3. Distinct differences exist in the skin lipids of males and females.
4. Samples obtained during the breeding season were qualitatively different from those acquired during the non-breeding season.

## INTRODUCTION

Skin lipids are known to serve multiple functions in the class Reptilia. Previous investigators have identified lipids in the skin secretions of both snakes and lizards (Ahern and Downing, 1974; Jackson and Sharaway, 1978; Tsumita *et al.*, 1979; Roberts and Lillywhite, 1980; Birkby *et al.*, 1982; Burken *et al.*, 1985a,b; Schell and Weldon, 1985). Most of these studies have focused on the role skin lipids play in retarding transepidermal water loss (Roberts and Lillywhite, 1980; Lillywhite and Maderson, 1982; Baeyens and Rountree, 1983; Burken *et al.*, 1985b). A few studies have attempted to document the variation in lipid components across different taxa of snakes. Schell and Weldon (1985) utilized $^{13}$C-NMR to ascertain the skin components of several genera of snakes. Burken *et al.* (1985) analyzed extensively the polar and non-polar skin lipids of 24 species of snakes by TLC. Both of these studies showed both similarities and differences in the skin lipids between species.

Skin lipids also serve as semiochemicals involved in chemical communication. Noble (1937) hypothesized that skin-derived chemical cues mediated courtship behavior in garter snakes. Greenberg (1943) also surmised the importance of epidermal chemical cues in the reproductive behavior of the Western banded gecko, *Coleonyx variegatus bairdi*. Mostly recently, Garstka and Crews (1981) investigated the sex attractant pheromone of the red-sided garter snake, *Thamnophis sirtalis parietalis*.

Female garter snakes produce a sex attractant pheromone that elicits courtship behavior from sexually active males. This pheromone is expressed along the female's dorsal surface and does not appear to have a discrete glandular source. In an earlier study, (Mason and Crews, 1985) we demonstrated that hexane washes of sexually attractive female garter snakes would elicit courtship behavior from sexually active male garter snakes in both field and laboratory trials. These results suggest that the sex attractiveness pheromone is non-polar in nature and probably in the lipid class. We have begun the chemical isolation and identification of this sex recognition pheromone in the red-sided garter snake, *Thamnophis sirtalis parietalis*. We report here the initial findings of our analysis of skin lipids of both male and female red-sided garter snakes.

## MATERIALS AND METHODS

### *Collection of skin lipids*

All animals used in this research were field collected in the spring and fall of 1985 near Narcisse, Manitoba, Canada. The extraction process involved soaking males and females for 12 hr in hexane. The animal was killed with an overdose of Brevital sodium and placed in the bottom of a 500-ml beaker so that its dorsal surface was on the bottom of the beaker. Approximately 5–10 ml of pesticide grade hexane was then poured into the beaker. Care was taken to ensure that the head and cloaca were not in contact with the solvent so that bodily fluids did not contaminate the wash. The beaker was covered with aluminium foil and sealed with Parafilm. After 12 hr the hexane wash was removed from the beaker was covered with aluminum foil and sealed with Parafilm. After 12 hr the hexane wash was removed from the until use.

### *Preparation of sample*

Hexane washes from each individual animal were filtered through a scintered glass funnel. This procedure removed particulate matter and some inorganics present in the snake's skin. The samples were pooled in groups of five animals each and then reduced to a residue in a rotary evaporator under vacuum at 50°C. The residue was then taken up in 1.0 ml of fresh hexane and subjected to analysis.

### *Thin-layer chromatography*

For TLC, a concentration of 25 mg/ml (sample/solvent) was used. Eight to ten microlitres aliquots were applied to the plates. The plates were 20 × 20 cm silical gel G plates with a 250 $\mu$m layer from Analtech. The plates were developed in hexane:ethyl ether:ethyl acetate (125:5:3). The solvent system was added to glass chromatography tanks with filter paper along the long sides to aid in equilibration. The tank was sealed with starch paste and allowed to equilibrate for 1 hr. The plates were added and allowed to develop to 18 cm. The plates were removed and allowed to air dry. The resulting chromatograms were sprayed with 50% $H_2SO_4$ and heated to 150°C for 1 hr.


Fig. 1. Thin-layer chromatograms of skin lipids of sexually active male and female red-sided garter snakes, *Thamnophis sirtalis parietalis*. Lane on the left contains the following lipid standards: CH, cholesterol; OA, oleic acid; TR, triolein; MO, methyl oleate; CO, cholesteryl oleate. The middle lane contains the males' skin lipids while the right lane contains the females' skin lipids.

*Gas chromatography/mass spectrometry*

Gas chromatography was performed on a Finnigan-MAT 4920 GC with a quadrupole mass spectrometer. The system is supported by the INCOS data system and includes a library containing over 33,000 spectra. The column was a 12-m fused silica BP-5 (0.25 mm i.d./0.25 film) capillary column from Scientific Glass Engineering. One microliter samples (25 mg/ml hexane) were injected onto the column using a 10-sec grob injection. The carrier gas was helium with a flow rate of 20 p.s.i. The injection temperature was set at 270°C and the ion source on the mass spectrometer at 150°C. The oven was held at 60°C for 1 min and then programmed to ramp up to 290°C at 10°C/min. The mass spectra were obtained under electron impact conditions.

## RESULTS

The charred chromatogram of the TLC is shown in Fig. 1. The lipid standards are included for comparison. The chromatogram shows distinct sex differences in the skin lipids of males and females. However, TLC does not allow us to determine the identity of the bands from the charred plates. We thus ran similar samples on GC/MS in order to identify the compounds present in the skin washes.

The GC trace in Fig. 2 again shows the distinct sex difference in the skin lipid profile of males and females during the breeding season. Figure 3 is a GC trace of the skin lipids from males with the accompanying mass spectral data. The MS data indicate the presence of fatty acids and cholesterol. The two fatty acids were well resolved and in sufficient quantity to assign structures. Thus, the peak at 874 was identified as hexadecanoic acid and the peak at 986 as octadecadienoic acid. The cholesterol peak at 1427 shared an identical retention time with pure cholesterol.

A distinctly different MS profile was observed in the females' skin lipids (Fig. 4). Again, the presence of cholesterol is noted at 1419 in the GC trace. There are also two other peaks whose base peaks and fractionation patterns suggest a steroid conformation. The peak at 1424 possesses a base peak and fractionation pattern similar to androstanediones. The peak at 1441 was not detected in high enough quantity to elucidate structures. These two peaks must be isolated and purified in order to elucidate them any further.

The skin lipids of male and female garter snakes in non-breeding condition are depicted in Fig. 5. Here we see the continued presence of the cholesterol peaks in both the male and female traces. However, the fatty acids were not present in the males' trace while the two steroids peaks are absent from the females' trace.

## DISCUSSION

The data in this paper demonstrate that there are clear sex and seasonal differences in the skin lipids of male and female garter snakes. In addition, these data suggest that GS/MS is a viable technique for resolving at least some of the neutral and non-polar lipids contained in reptilian skin.

The presence of cholesterol in the skin lipids of snakes has been reported previously (Ahern and Downing, 1974; Jackson and Sharaway, 1978; Roberts and Lillywhite, 1980; Burken *et al.*, 1985b; Schell and Weldon, 1985). Thus, the presence of cholesterol in the GC traces of both males and females validates this technique.

Burken *et al.* (1985b) did not specifically look for sex differences in the skin lipids of the snakes they examined. However, they thought that their preliminary indications were that sex differences were not a factor. We have shown here that in the red-sided garter snake, *Thamnophis sirtalis parietalis* there is a profound difference in the pooled skin lipids of males and females. Presumably, some portion of the females' skin lipids has a chemosensory function (Garstka and Crews, 1981; Mason and Crews, 1985). It is interesting that the skin lipids of both male and female garter snakes change during the non-breeding season both quantitatively and qualitatively. This seasonal difference in skin lipids may be responsible for the extinction of male courtship after the brief 3–4

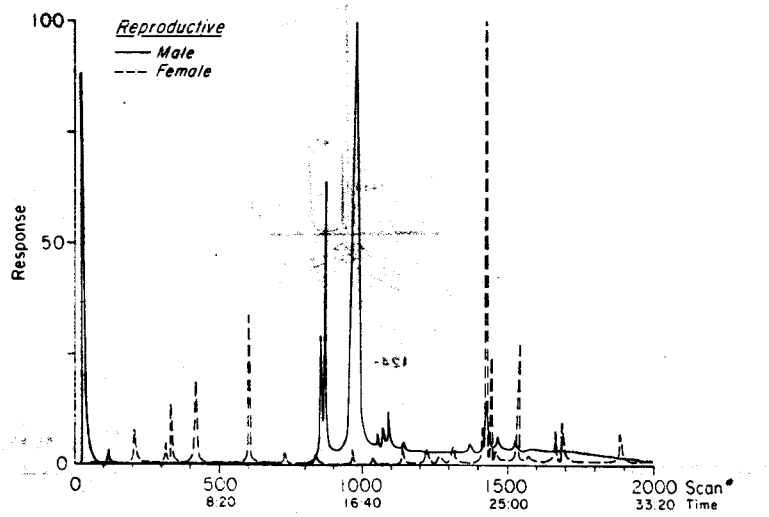


Fig. 2. Gas chromatograms of skin lipids of male and female red-sided garter snakes, *T. s. parietalis* acquired during the breeding season (retention times in min).

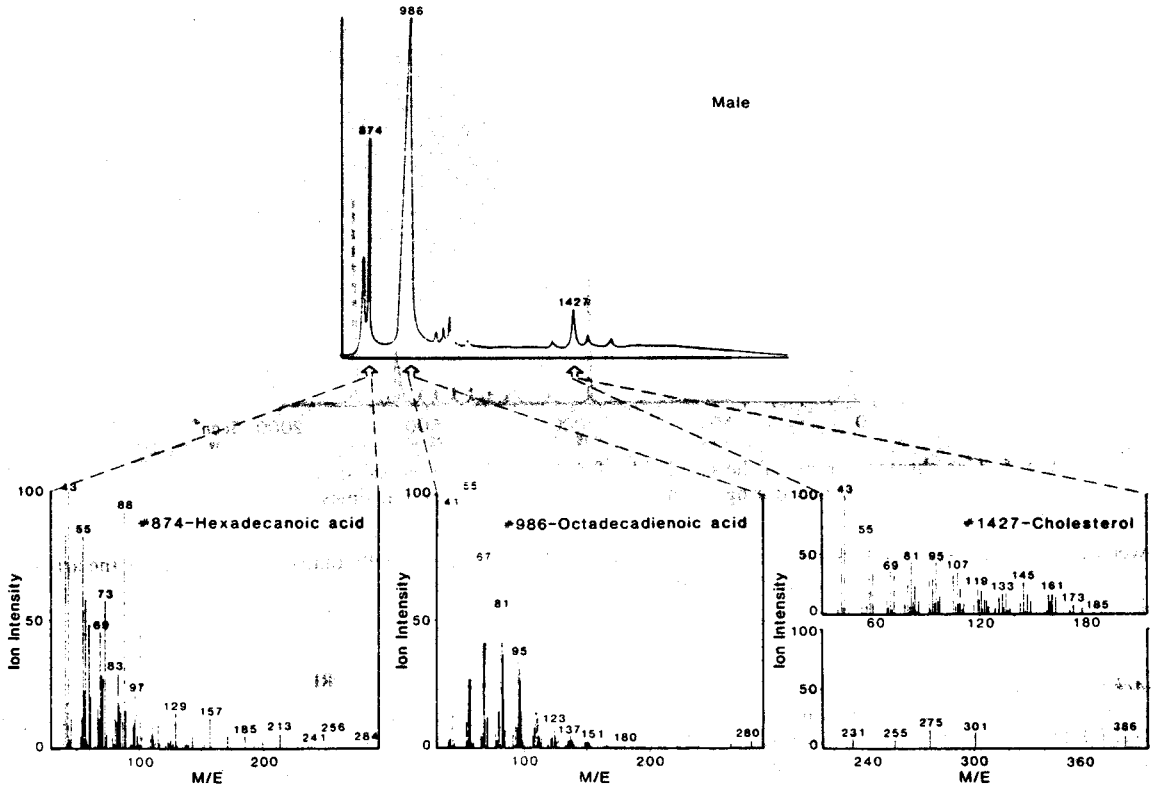


Fig. 3. Gas chromatogram of hexane skin washes of sexually active male red-sided garter snakes, *T. s. parietalis*, with corresponding mass spectral data (retention times in min).

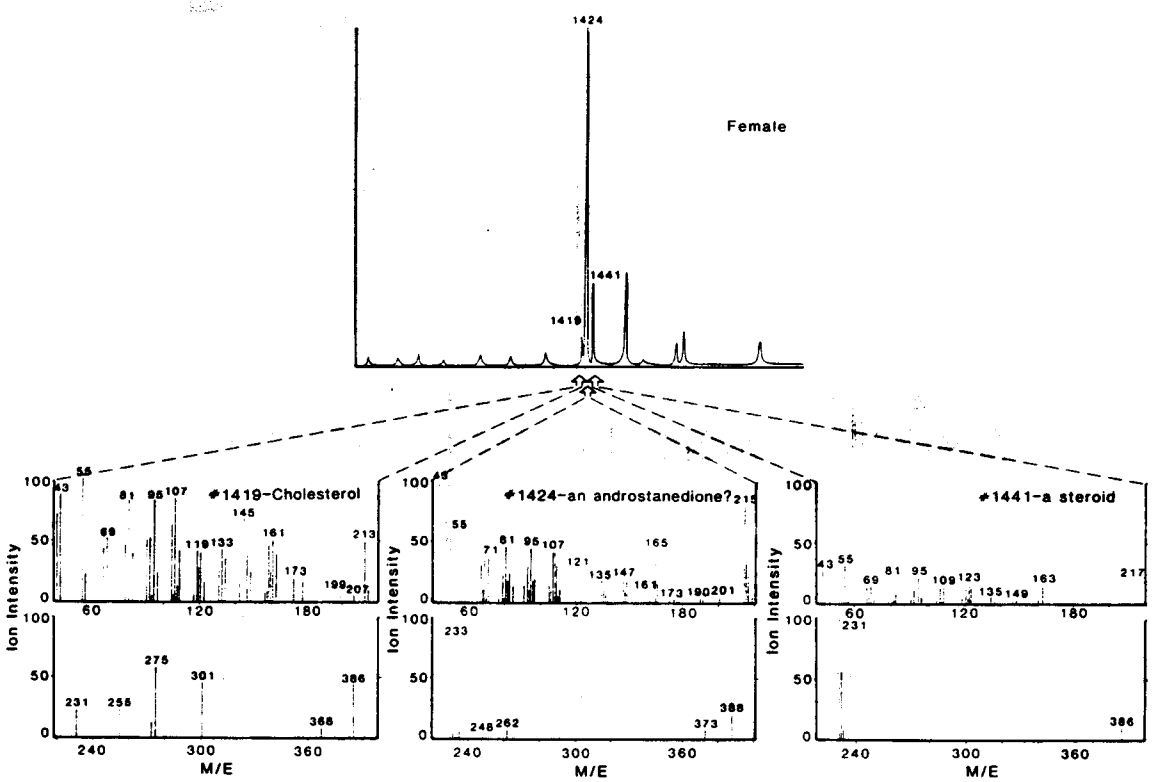


Fig. 4. Gas chromatogram of hexane skin washes of sexually active female red-sided garter snakes, *T. s. parietalis*, with corresponding mass spectral data (retention times in min).

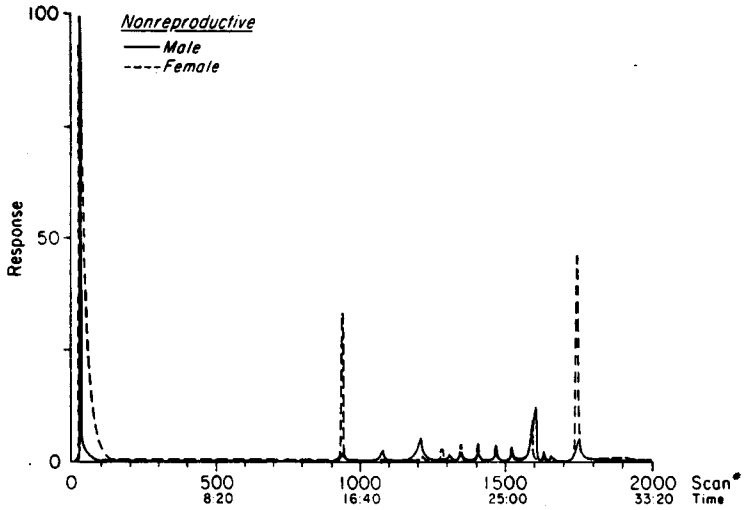


Fig. 5. Gas chromatograms of the skin lipids of male and female red-sided garter snakes, *T. s. parietalis* obtained during the non-breeding season (retention times in min).

week breeding season. We are currently attempting to isolate and identify the compound(s) serving as the sex recognition pheromone in this species.

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