

REPRODUCTIVE ISOLATING MECHANISMS BETWEEN TWO SYMPATRIC SIBLING SPECIES OF SEA SNAKES

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Abstract.—Mechanisms that maintain species isolation within sympatric congeners have attracted analysis in many kinds of organisms, but not in snakes. We studied two sibling species of amphibious sea snakes (*Laticauda colubrina* and *L. frontalis*) on the island of Efate, in the Pacific Ocean republic of Vanuatu. The two taxa are almost identical morphologically, except that *L. colubrina* grows much larger than *L. frontalis*. No natural hybrids have been reported, and geographic distributions of the two taxa suggest the possibility of sympatric speciation. Our fieldwork shows that the two taxa are often syntopic and overlap in breeding seasons. Behavioral studies in outdoor arenas show that the separation between these two taxa is maintained by species-specific cues that control male courtship. Males of both species courted conspecific females but not heterospecific females. The proximate mechanism driving this separation involves chemical cues. Adult females of both taxa possess distinctive lipids in the skin. Males directed courtship behavior (chin-pressing) to hexane-extracted samples of lipids from conspecific but not heterospecific females. Males of the dwarf species (*L. frontalis*) were more selective courters than were those of the larger taxon (*L. colubrina*), perhaps because a preference for courting larger females means that *L. colubrina* males would be unlikely to court *L. frontalis*-sized (i.e., small) females even in the absence of pheromonal barriers.

Key words.—Courtship, *Laticauda*, pheromones, sea krait, speciation.

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Within many phylogenetic lineages, it is common to find situations where sibling taxa, closely related and similar in most aspects of morphology, physiology, and behavior, exist in sympatry. How do such taxa maintain their distinctiveness instead of hybridizing back into a single taxon? Darwin (1859) identified this question as one of the most fundamental issues in evolutionary biology, and the topic has received extensive study since that time. An array of species isolating mechanisms have been identified, some that prevent mating and some that act after mating to prevent the production of viable hybrids (e.g., Mayr 1972; Andersson 1994). In animals, behavioral factors are generally the most important in preventing interspecific matings, and an extensive literature on species recognition mechanisms documents an array of examples whereby such discrimination is achieved (Dobzhansky 1951; Tregenza et al. 2000).

The potential role of sexual selection in generating speciation either in sympatry (Panhuis et al. 2001; Via 2001) or peripatry (Odeen and Florin 2002) has stimulated a recent resurgence of interest in reproductive interactions between sympatric taxa. However, a strong taxonomic bias is apparent in the species used to study these questions. For example, we scored the organisms mentioned in tables as exemplars of speciation studies in a recent major review of speciation research (*Trends in Ecology and Evolution*, vol. 16, no. 7, 2002). Of the more than 100 cited taxa, none were reptiles. Phylogenetic diversity among case studies of this topic is crucial, because the sensory modalities involved in interspecific discrimination vary among lineages. For example, visual displays produce species-specific responses in some lizard taxa (Losos 1985). Pheromonal differences among sympatric species may provide the basis for species recognition and avoidance of interspecific matings in other lizards (Cooper and Vitt 1984, 1987), as occurs in many other kinds of an-

imals (e.g., insects: Higgin et al. 2000). Although snakes have attracted very little research in this respect, it seems likely that chemical cues will provide the most important species isolating mechanisms. Pheromonal communication is clearly the most important cue in courtship and mating (Noble 1937; Mason 1992; Weldon et al. 1992), and in at least one lineage, males have been reported to trail-follow conspecific rather than heterospecific females (Ford 1982; Ford and Schofield 1984). Nonetheless, there have been no studies of the actual mechanisms by which sympatric snake species refrain from interbreeding. The question is particularly interesting for marine snakes, because skin lipids in snakes serve for waterproofing as well as sex pheromones (Mason 1992). Convergent natural selection for waterproofing in these semiaquatic animals might thus constrain interspecific divergence in sex-recognition signals.

We studied species isolating mechanisms in a system far removed, both geographically and phylogenetically, from those used in any previous studies. Laticaudine sea snakes are abundant in many parts of the Pacific Ocean, and come ashore to court and mate on small islands in this region (Voris and Voris 1995; Heatwole 1999). Closely related laticaudine species live in sympatry in several regions, and can be found coiled together under shelter items in such areas. Hybrids have never been reported in the taxonomic literature (H. G. Cogger, pers. comm.). Why don't these taxa interbreed, given their syntopy and close relatedness? Species isolation could be maintained by a lack of contact between the two sibling taxa, either in space or time. Thus, we evaluated whether the two taxa inhabit the same places and whether they breed at the same time of year. Because we found substantial overlap in both attributes, we then set out to determine whether behavioral mechanisms might be responsible for species isolation and, if so, what kinds of cues were involved.

MATERIALS AND METHODS

The Pacific nation of Vanuatu lies 170 km south of the Solomon Islands, 800 km west of Fiji, and 230 km northeast of New Caledonia. We studied laticaudine sea snakes on the northeastern tip of the island of Efate, near Paonangisu village (Nagar Resort: 17°35'S, 168°29'E). The climate is tropical; mean air temperature is >26°C in most months. Rainfall is concentrated in the months from December to February (O'Byrne and Harcombe 1999).

Three laticaudine species are found in Vanuatu, all belonging to a single clade with highly specialized dietary habits (feeding only on moray and conger eels; Greer 1997). One taxon, *L. laticaudata*, differs strikingly in body shape and coloration from the others. However, the two remaining taxa are so similar that until recently they have been treated as a single species, even after detailed taxonomic analysis (McCarthy 1986). Between 1983 and 1996 extensive field and laboratory research on laticaudine sea kraits was conducted in the western Pacific under the leadership of Professor Nobuo Tamiya (Tohoku University) and Dr. Toru Tamiya (Sophia University). One outcome of this research was the discovery that populations of the yellow-lipped sea krait (*Laticauda colubrina*) in Vanuatu actually comprised two syntopic sibling species: the larger *Laticauda colubrina* (*s.s.*) and a much smaller sister species, for which the name *Laticauda frontalis* (De Vis 1905) is available (Cogger et al. 1987). Thus, there are two sibling species within this clade. One is a large (to >1.5 m) species, *L. colubrina*, that is widespread through the Pacific from Fiji in the east to Sumatra in the west (McCarthy 1986; Heatwole and Guinea 1993). The other taxon (*L. frontalis*) is smaller (<1.0 m) and is endemic to Vanuatu. Although *L. frontalis* has been synonymized with *L. colubrina* in virtually all published literature, recent studies by H. G. Cogger and H. Heatwole (unpubl. ms.) show that *L. frontalis* is clearly distinct from *L. colubrina* in several aspects. For example, counts of ventral scales do not overlap between the two taxa, and *L. frontalis* attains much smaller maximum sizes than does *L. colubrina* (80 vs. 140 cm snout-vent length [SVL] in Vanuatu; Shine et al. 2002). Because of the close relationship between *L. colubrina* and *L. frontalis*, these two species were the subjects for our research on species isolating mechanisms.

Both *L. colubrina* and *L. frontalis* are black-and-white banded snakes with flattened paddle-like tails. The most convenient field-identification method involves a subtle difference in pattern: the black band encircling the head is in contact with the first body band in *L. colubrina* but not in *L. frontalis*. There have been no previous studies of the ecology or behavior of sea snakes in Vanuatu, and thus *L. frontalis* has not attracted any previous research. However, the wide-ranging *L. colubrina* has been intensively studied in other parts of its geographic range. Radiotelemetric studies on a Fijian population of *L. colubrina* found that these snakes spent about half of their time at sea (foraging for eels) and the other half on land (digesting prey, sloughing, courting, and mating). They moved between the two habitats about once every 10 days (Shetty 2000; Shetty and Shine 2002b).

Laticaudine snakes may breed year-round in tropical areas, but with a nonreproductive period in cooler months in less

tropical climates (for a review see Greer 1997). Detailed research at a Fijian site containing one of our study species (*L. colubrina*) documented a peak in mating activity around November (Guinea 1986; Shetty and Shine 2002a). These data suggest that Vanuatu laticaudids will breed year-round, but with a peak around November. Thus, we visited Efate from 1 November to 15 December 2000.

We collected snakes by night (using a flashlight to find actively moving snakes, typically close to the land-water interface) and by day (when they were hidden inside small crevices in rocks or mangrove trees). We surveyed sites on the main island of Efate as well as on small outlying islands, but our main study sites were all within a 4-km circle: a sandy beach 2 km south of Paonangisu on the main island; a rocky shore on the offshore island of Kakula 1 km northwest of Paonangisu; two tiny rocky islets <1 km from Kakula; and a clump of mangrove trees <300 m from the islets. Collecting effort was spread among these sites throughout the 6-week-period of our study; we saw no evidence of any temporal shifts in species numbers at any of the sites.

All snakes were identified, weighed, and measured (SVL) and then given an individual scale-clip for identification. They were kept in cloth bags, in the shade, until needed for experimental studies. We used only adult animals for these studies (criteria for sexual maturity = for *L. colubrina*, male > 70 cm SVL, female > 90 cm SVL; for *L. frontalis*, male > 40 cm SVL, female > 50 cm SVL; Shetty and Shine 2002a,b; R. Shine et al. 2002). For detailed observations of courtship behavior, we erected five open-topped nylon arenas in a shady location. Each measured 1 m × 1 m × 1 m, and was supported by plastic rods. The base of each enclosure was filled with a 5-cm layer of beach sand, which was removed and replaced between trials to ensure that no pheromonal cues remained to confound snake responses.

We conducted two sets of experiments using these enclosures. In the first set, one adult male and one adult female snake were added to each enclosure and their subsequent behavior scored to compare the intensity of courtship that male *L. colubrina* and *L. frontalis* directed to conspecific versus heterospecific females. Only two snakes (one male, one female) were used in each trial, to eliminate the possibility of confounding by interactions with other snakes. Each snake was used only once. The trial was terminated as soon as we recorded intense courtship (body-jerking, head-pressing) or (failing this) after 8 h. Each hour, we scored the snakes' location and behavior in each arena. Because we terminated trials as soon as we saw active courtship, we did not record copulation in the arenas. However, *L. colubrina* will copulate in such enclosures (Shetty 2000; Shetty and Shine 2002a). We used logistic regression to analyze these data, with the dependent variable being the intensity of response (whether or not the male aligned his body with that of the female). This analysis was performed using Statview 5 (SAS Institute 1998) on a Macintosh G4 computer.

The second set of trials was designed to assess whether lipids in the skin of females served as sex pheromones to elicit courtship by males (as has been shown in garter snakes; Mason 1993) and whether such pheromones might act as cues for species isolation. To obtain skin lipids, we soaked squares of clean, soft cotton cloth (15 cm × 15 cm) in fresh water,

and then applied reagent-grade hexane (C_6H_{12}) to the middle of each cloth. Some of the cloth pieces were used as controls for the presence of hexane, whereas others were wiped across the dorsal surface of adult female snakes to obtain a sample of skin lipids (cf. Mason 1993). To do this, the female was held by the neck and hindbody so that her cloacal secretions could not contaminate the sample. The snake was first rubbed with a water-dampened cloth to remove sand and other impurities, and we then wiped the cloth four times along the female's back in an anterior-to-posterior direction. We used four female *L. colubrina* per cloth to obtain the stimulus for this species, and six females per cloth for *L. frontalis* (because of the smaller body size of this taxon).

We then placed three pieces of cloth (one hexane control, one with *L. frontalis* lipids, and one with *L. colubrina* lipids) in an enclosure containing four to seven adult male snakes of one of the species. Using multiple males within a single arena mimics the natural situation with breeding laticaudines (Guinea 1986; Shetty and Shine 2002a) and male-male competition does not modify responses because male laticaudines display no overt aggression toward each other in mating aggregations (Guinea 1986; Shetty and Shine 2002a). Indeed, males do not display any overt reaction to each other's presence whatsoever. Two replicate arenas were used for each species, to test a total of 13 male *L. colubrina* and nine male *L. frontalis*. Each male snake was scored for its response to all three of the cloth squares in its arena. Each animal was paint-marked with an identification number on its back, so that we could maintain separate records for each male. Initial tests suggested that moving the stimulus cloth was likely to disturb animals and thus might generate artifactual responses. In nature, female laticaudines generally remain still and are encountered by trail-following males (Shetty and Shine 2002a). Thus, we laid the cloth squares out on the floor of the arena, well-separated from each other, and waited until males happened across the stimuli. We recorded each male's response from the first time that it encountered each stimulus (i.e., when the male's head was directly above the cloth). A few animals that remained sedentary throughout this period, and therefore did not encounter any of the stimuli, were gently disturbed (nudged midbody with a stick) to stimulate activity. We then scored their behavior when they encountered one of the cloth pieces, in the same way as for the other snakes. No differences in responses to the cloth squares were apparent between the snakes that we stimulated to move versus those that were not stimulated in this way.

For each male, we recorded data only for the first time he encountered each of the cloth pieces. The responses that we scored were the number of tongue-flicks (complete cycles of tongue extrusion and withdrawal) before leaving the cloth and whether the male pressed his chin against the cloth after tongue-flicking. The number of tongue-flicks may reflect the male's overall level of interest in the stimulus, and the chin-press is a distinctive behavior seen only during male courtship (Gillingham 1987; Mason 1993; Shine et al. 2000a). These data were analyzed by repeated-measures ANOVA, with responses to each of the three cloth pieces serving as the repeated measure for each male.

After the behavioral trials were completed, we used hexane to extract samples of skin lipids from adult males and females

of all three Vanuatu laticaudine species. One male and female from each of the three species were killed with an overdose of brevitall sodium injected peritoneally. Each snake was then placed dorsal side down in a 2-L stainless steel bowl, 300 ml of hexane was added, and the bowl covered with aluminum foil and left for 12 h (Mason et al. 1989, 1990). Care was taken to keep the head and cloaca out of the hexane to avoid possible contamination by internal bodily fluids. After removal of the animals, the resulting hexane extracts were reduced in volume by evaporation and the remainder was transported back to the laboratory in Teflon-capped glass vials. Upon return to the laboratory, the excess solvent in the samples was removed by rotoevaporation at 35°C and the resulting lipid residues were weighed to the nearest milligram on a digital scale (Mettler AT400, San Francisco, CA). The residues were resuspended in fresh hexane (1–2 ml) and sealed in 9-ml glass vials with polyethylene-lined caps and stored at –20°C.

Hexane-extracted skin lipids were further analyzed by gas chromatography/mass spectrometry (GC/MS). This was accomplished with a Hewlett-Packard (Palo Alto, CA) 5890 Series II gas chromatograph fitted with a split injector (280°C) and a Hewlett-Packard 5971 Series Mass Selective Detector. Aliquots (1 μ l) of the extracts were injected onto a fused-silica capillary column (HP-1; 12 m \times 0.22 mm I.D.; Hewlett-Packard) with helium as the carrier gas (5 cm/sec). Oven temperature was initially held at 70°C for 1 min, increased to 310°C at 10°C/min and finally held at 310°C for 10 min. Resulting peaks were identified by ChemStation software (ver. B.02.05, Hewlett-Packard) interfaced with the GC/MS, comparison to known compounds or comparison to library spectra.

RESULTS

Do the Two Species Exist Syntopically?

Adults of both laticaudine species were recorded at three of the four sites where we collected, with *L. frontalis* always less common than *L. colubrina* (nine vs. 53 specimens on the main island of Efate; 10 vs. 30 on the offshore island of Kakula; three vs. 19 on tiny offshore islets). In a small clump of mangrove trees, we found 14 adult *L. colubrina* (and 15 juveniles of this species) but no *L. frontalis*. Contingency-table analysis revealed no significant difference in the relative distributions of adults of the two species ($\chi^2 = 5.29$, $df = 3$, $P = 0.15$). More extensive surveys might well reveal interspecific differences in distribution, but our data show extensive overlap between the locations of adult *L. colubrina* and adult *L. frontalis*. We often found specimens of the two taxa within a few meters of each other. Thus, the two species must regularly encounter each other, at least at this time of year.

Do the Two Species Breed at the Same Time of Year?

We recorded overt courtship in the field only once. A male *L. colubrina* was seen lying on and actively courting an adult female of the same species at the mainland site near Paonangisu. We also recorded five male-female pairs of *L. colubrina* in close proximity to each other. We did not see field

courtship in *L. frontalis* but recorded vigorous courtship in this species in arena trials. In three cases, males were fully aligned with the female's body and engaged in courtship behaviors (body-jerking, head-pressing) that were superficially identical to those of *L. colubrina* (Shetty and Shine 2002a).

What Cues Elicit Courtship by Males?

Observations of courtship in arena trials, as well as our previous experience with field courtship and copulation in *L. colubrina* (Shetty 2000; Shetty and Shine 2002a,b), strongly suggest that contact pheromones are the most important cues for courtship in laticaudine snakes. Trail-following and courting males tongue-flick frequently, and a male encountering a female repeatedly tongue-flicks her dorsal surface as he crawls forward and aligns his body with hers. As in most other snake species (Gillingham 1987), the courting male then presses his chin firmly against the female's dorsum while intermittently jerking his entire body and attempting to lift her tail upward with his own. Both the tongue-flicking and chin-pressing behaviors suggest that the vomeronasal system is involved in male courtship in sea kraits, as it is in other snakes (Noble 1937; Shine et al. 2000c).

Do Adult Female Sea Kraits Possess Pheromones in the Skin?

Courting male garter snakes (*Thamnophis sirtalis*) can distinguish the sex of other individuals via sex differences in skin lipids (Mason 1992). Mass spectrophotometry confirmed that sea kraits also have lipids on their body surface, and that the composition of these lipids differs between conspecific males and females (Fig. 1). Although cholesterol gave a large peak in each sample (at 22 min), sex differences in other lipids were evident in both species (Fig. 1).

Do the Female's Skin Pheromones Differ between the Two Species?

Lipids on the body surface of adult female *L. frontalis* differed considerably from those of adult female *L. colubrina* (Fig. 1), potentially offering cues for species as well as sex recognition. Lipid profiles of *L. laticaudata*, the other Vanuatu sea-krait taxon, were broadly similar to those of *L. colubrina* (Fig. 1).

Do Males Preferentially Court Conspecific Females?

Snakes of both species settled down in the arenas, and we saw active courtship on several occasions. The arena trials revealed that males of both *L. colubrina* and *L. frontalis* exhibit a strong preference for conspecific rather than heterospecific females. Male *L. colubrina* aligned with female *L. colubrina* in 14 of 21 trials (67%) versus none of 10 trials with female *L. frontalis* (log-likelihood ratio from logistic regression with species of female as the independent variable and body alignment as the dependent variable: $\chi^2 = 15.95$, $df = 1$, $P < 0.0001$). Similarly, male *L. frontalis* aligned their bodies with those of conspecific but not heterospecific females (eight of 12 vs. zero of 11: log-likelihood ratio test, $\chi^2 = 14.44$, $df = 1$, $P < 0.0001$). Thus, males of each species

recognize conspecific females and do not court females of the sibling species.

Is Species Discrimination by Courting Males Based on Pheromonal Cues?

The male snakes responded in consistent ways to the three treatments that we offered on hexane-soaked cloth inside the arenas. Repeated-measures ANOVA on the tongue-flick responses of male snakes revealed that male *L. colubrina* were more responsive overall than were male *L. frontalis* ($F_{1,40} = 5.57$, $P < 0.03$) and that some stimuli attracted more tongue-flicks than did others (repeated measure, $F_{2,40} = 14.09$, $P < 0.0001$; post hoc tests show that the hexane control elicited fewer tongue-flicks than did either of the snake stimuli). Also, males of the two species responded to the various stimuli in different ways ($F_{2,40} = 12.46$, $P < 0.0001$). Male *L. colubrina* tongue-flicked most to the scent of female *L. colubrina*, whereas male *L. frontalis* tongue-flicked almost exclusively to the scent of female *L. frontalis* (Fig. 2).

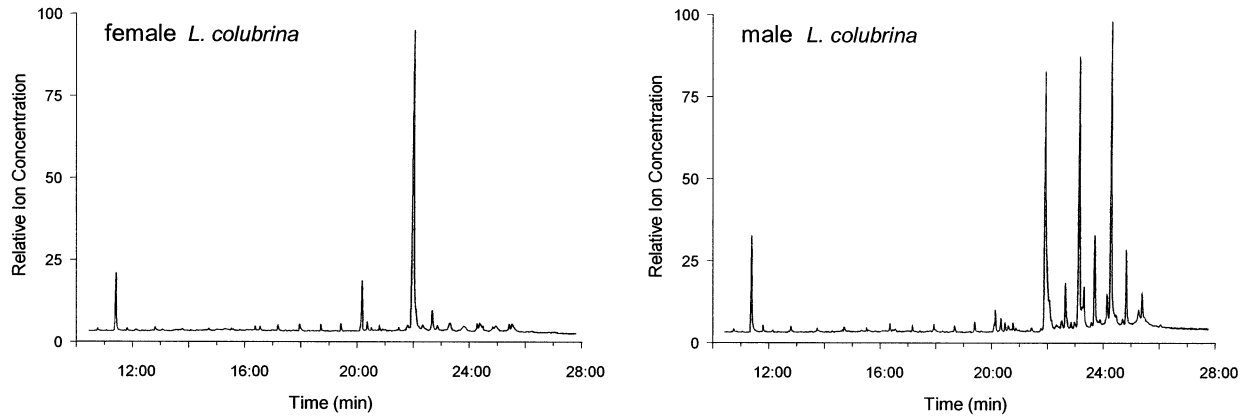
Logistic regression on the chin-pressing behavior of our test snakes also revealed significantly different responses to different stimuli. Chin-pressing was seen in nine of 13 trials when a male *L. colubrina* encountered the scent of a female *L. colubrina*, and in five of 9 trials when a male *L. frontalis* encountered the scent of a female *L. frontalis*. This behavior was never seen in any other context (heterospecific or control scent) and thus, male snakes of both species displayed chin-pressing more often to conspecific scent than to other treatments (log-likelihood ratio test, $\chi^2 = 26.09$, $df = 2$, $P < 0.0001$ for *L. colubrina*; $\chi^2 = 13.51$, $df = 2$, $P < 0.002$ for *L. frontalis*).

DISCUSSION

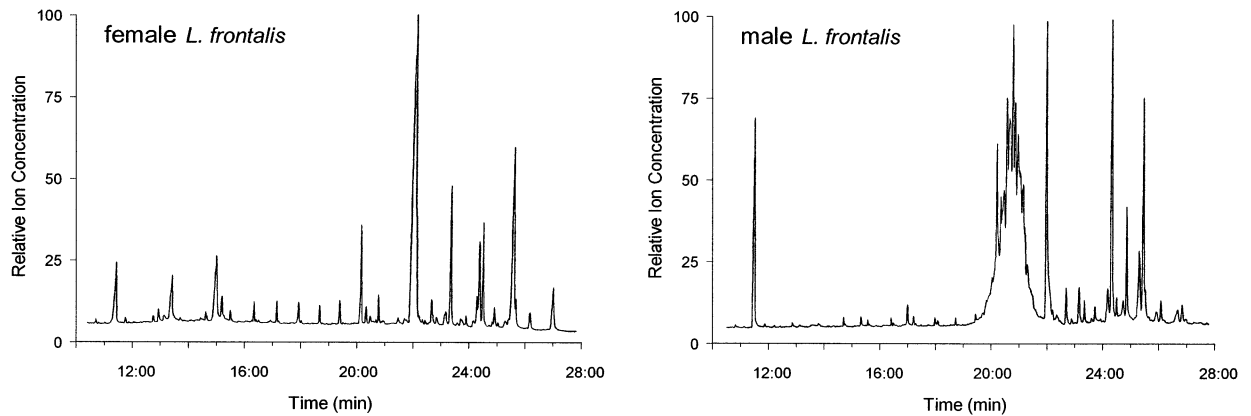
The two taxa that we have studied are so similar morphologically that they have long been considered conspecific (McCarthy 1986). Under any phenetic or cladistic analysis, these taxa form a single strongly supported clade within laticaudine phylogeny (e.g., Cogger et al. 1987; Heatwole and Guinea 1993; Greer 1997). Although these two species live in syntopy and breed at the same time of year (or at least, overlap substantially in this respect), hybrids have not been reported (based on a large dataset on morphology: H. G. Cogger, pers. comm.). The limited distribution of *L. frontalis*, entirely within the broader distribution of *L. colubrina*, raises the intriguing possibility that speciation in this lineage may have occurred through sympatric or peripatric processes (Panhuis et al. 2001; Odeen and Florin 2002). Our study suggests that the current separation between the taxa is maintained by species-specificity in the cues that elicit courtship by males of both species. Skin lipids act as sex pheromones in laticaudines, as in other snake species (Mason 1992; Welton et al. 1992), and interspecific differences in the skin-lipids of adult females are the means by which males are able to discriminate between conspecific and heterospecific females. Thus, selective pressures associated with the marine habitat of laticaudids have not prevented interspecific divergence in skin lipids sufficient to permit species discrimination by courting males.

The scarcity of field observations on reproductive behavior

A



B



C

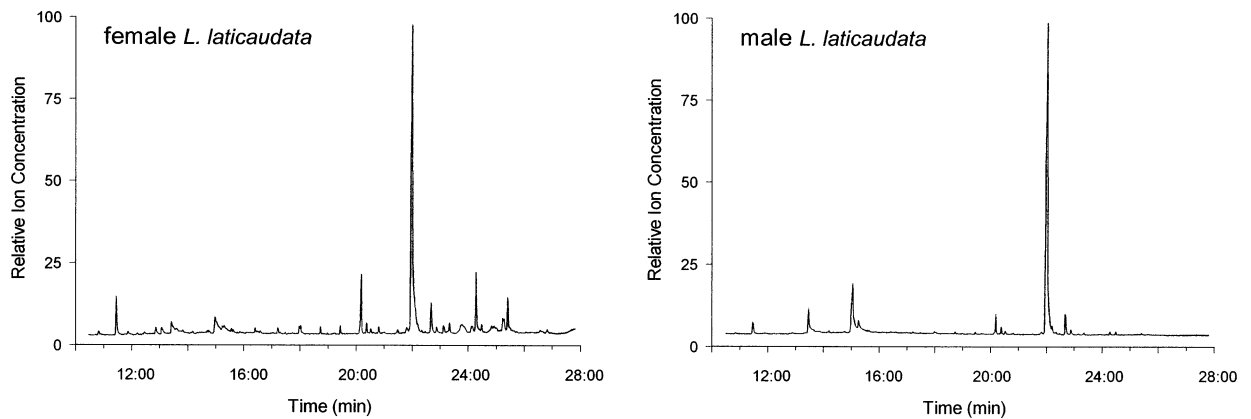


FIG. 1. Lipid profiles from hexane washes from three species of sea kraits, *Laticauda* spp., from Vanuatu. See text for methods used to obtain these profiles.

of Vanuatu sea-snakes means that we cannot be certain that the two species court and mate in the same places at the same times. However, extensive data on one of these taxa elsewhere in their range (*L. colubrina* in Fiji) indicate that courtship occurs over a relatively long period and in a wide variety

of habitats—apparently, wherever and whenever an adult male encounters an adult female (Shetty 2000; Shetty and Shine 2002a,b). Thus, we would expect occasional hybridization between *L. colubrina* and *L. frontalis* in the absence of behavioral barriers to mating.

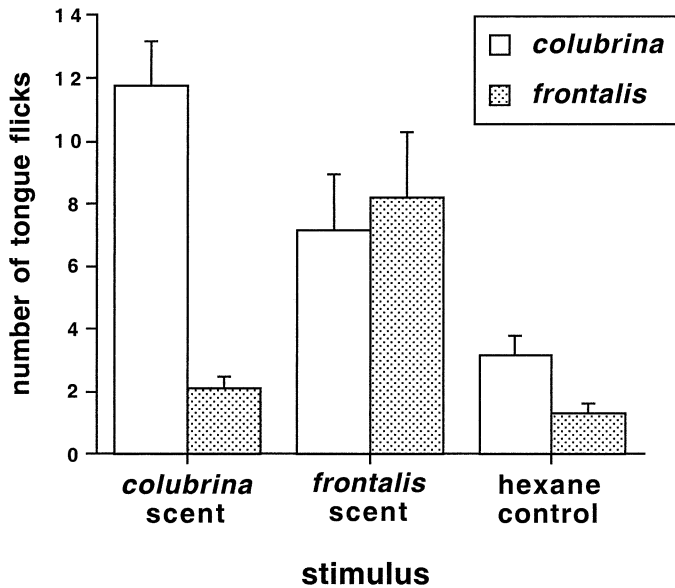


FIG. 2. Numbers of tongue-flicks exhibited by male sea snakes in response to three stimuli in arena trials. The male snakes belonged to two sibling species (*Laticauda colubrina* and *L. frontalis*) and the stimuli were squares of cloth soaked in hexane (control) or soaked in hexane and then wiped on the dorsal surfaces of adult females of either snake species. Graph shows mean value \pm one standard error. See text for statistical tests and further explanation.

In the outdoor arenas, males of both taxa commonly exhibited courtship to females of their own species, but never courted females of the other species. Although males also exhibited a strong species-specificity in their response to female lipids, male *L. colubrina* were more reactive and less discriminating in this respect than were male *L. frontalis* (Fig. 2). This result may be due at least partly to our experimental design. Because female *L. colubrina* are larger than female *L. frontalis*, our hexane-extracted samples may have contained a higher concentration of *L. colubrina* lipids than *L. frontalis* lipids. We attempted to control for this size difference by wiping six *L. frontalis* and only four *L. colubrina*, but nonetheless a difference may still have existed. If so, male *L. colubrina* in the pheromone trials may have been exposed to higher concentrations of conspecific pheromones, and thus (perhaps) reacted more strongly to all stimuli that they encountered.

Nonetheless, bias from our experimental procedures cannot explain the greater species specificity of *L. frontalis* than of *L. colubrina* males (Fig. 2). Male *L. frontalis* showed very few tongue-flicks to the scent of female *L. colubrina*, whereas male *L. colubrina* tongue-flicked frequently to the scent of female *L. frontalis* (Fig. 2). Why should male *L. frontalis* be more selective than male *L. colubrina* in this respect? There are two plausible answers to this question. The first is that *L. colubrina* is much more common than *L. frontalis*, so that most of the female snakes that male *L. frontalis* encounter would be the “wrong” species. These males should thus be under more intense selection for species recognition than would male *L. colubrina*, which encounter the wrong species of female less often. This asymmetry may be exaggerated by another cue that male *L. colubrina* use for courtship: female

body size. In arena trials with Fijian *L. colubrina*, males devoted much more intense courtship to large females than to smaller animals (Shetty and Shine 2002a). The same pattern has been documented in American garter snakes, and may reflect the greater fecundity of larger females (Hawley and Aleksasuk 1975). In the case of our two laticaudine species, a preference for larger females means that male *L. colubrina* would be unlikely to court female *L. frontalis*. The largest female *L. frontalis* in our sample (78.3 cm SVL, 176 g) was smaller than any of the adult female *L. colubrina* (96.3 cm SVL, 261 g; Shine et al. 2002).

It would be interesting to know if male *L. frontalis*, like male *L. colubrina* and male garter snakes, prefer to court and mate larger rather than smaller females. Such a pattern would generate even more intense selection for species recognition in *L. frontalis* than in *L. colubrina*. In the absence of pheromonal barriers, a male *L. colubrina* is unlikely to court a (small) female *L. frontalis*; but in the absence of such barriers, a male *L. frontalis* would presumably court female *L. colubrina* (indeed, these might offer a super-stimulus). Our data from the hexane-extract trials fit well with this scenario, in suggesting that male *L. frontalis* are more selective courters than are male *L. colubrina* (Fig. 2). Such asymmetries may be common in species isolating mechanisms (Arnold et al. 1996; Tregenza et al. 2000).

Asymmetries may also influence selection for distinctive traits in females. Because *L. frontalis* is much less common than *L. colubrina*, female *L. frontalis* may have more chance of being courted by the wrong males (unless size is a major factor; see above). Thus, they may benefit from having unambiguous species-identifiers that discourage courtship by male *L. colubrina*. Our data on skin lipids fit well with this speculation. Female *L. frontalis* display several lipid components not found in female *L. colubrina* (Fig. 1). Intriguingly, some of these are similar to lipids in male *L. colubrina*, and (by analogy with garter snakes; Mason and Crews 1986) may tend to discourage courtship by such males. The similarity between lipid profiles in females of *L. colubrina* and *L. laticaudata* suggest that it is *L. frontalis* that has evolved differences in this respect (Fig. 1).

Although barriers to hybridization have been explored in many taxa (Howard and Berlocher 1998), reptiles have attracted relatively little research on this topic. The most detailed work involves lizards, where reproductive isolation seems to depend upon visual cues in some taxa (Losos 1985) and chemical cues in others (Cooper and Vitt 1987). In snakes, Ford (1982) and Ford and Schofield (1984) have shown that male garter snakes preferentially follow pheromonal trails deposited by females of their own rather than other species. However, this work did not include courtship behavior.

This lack of research on species isolating mechanisms in snakes is surprising, because the mating systems of several snake species facilitate such work. For example, many natricine taxa live in large mixed-species aggregations during the mating season, and experimental studies clearly demonstrate the importance of skin lipids as contact pheromones in such systems (Mason and Crews 1986; Mason 1993). A lineage such as the American garter snakes (*Thamnophis*) offers an ideal opportunity to explore the evolution of re-

productive isolation by examining the ways in which female lipids, and male responses to those lipids, have changed through phylogeny (Ford 1982). Studies on visual and auditory mate-recognition systems in other kinds of organisms have documented a range of evolutionary phenomena such as sexual selection for sensory exploitation of existing sex-recognition systems (Ryan et al. 1990). Given the evolution of pheromonally based female mimicry in at least one snake species (Mason and Crews 1986; Shine et al. 2000a,b), phylogenetic shifts in female lipids and male responses within snake lineages provide an exceptional opportunity to explore the evolution of mate recognition systems.

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